

Hepatitis C virus (HCV) genome organization.

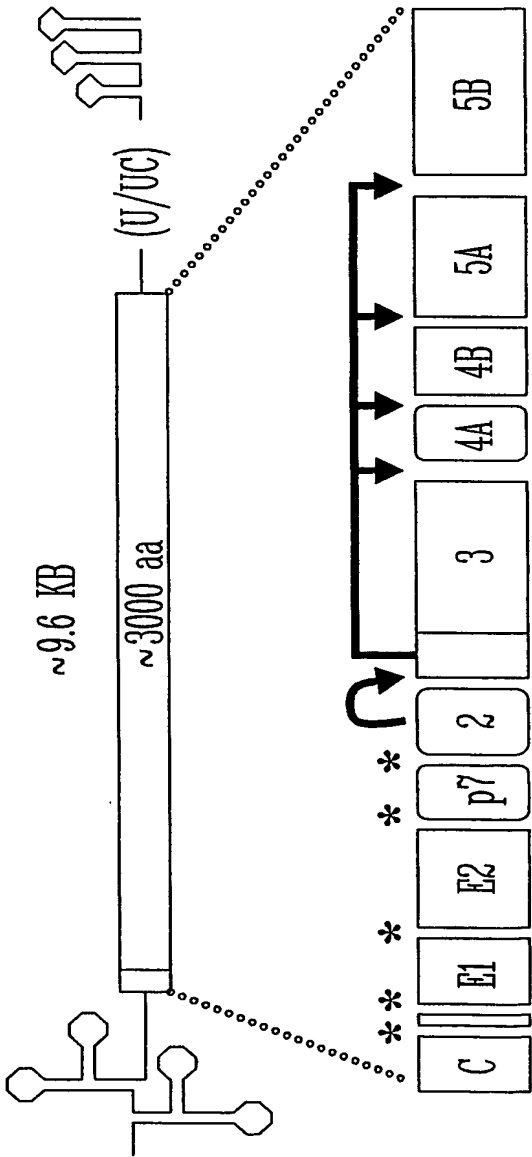


FIG. 1

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# Hypothetical model of the HCV replication cycle

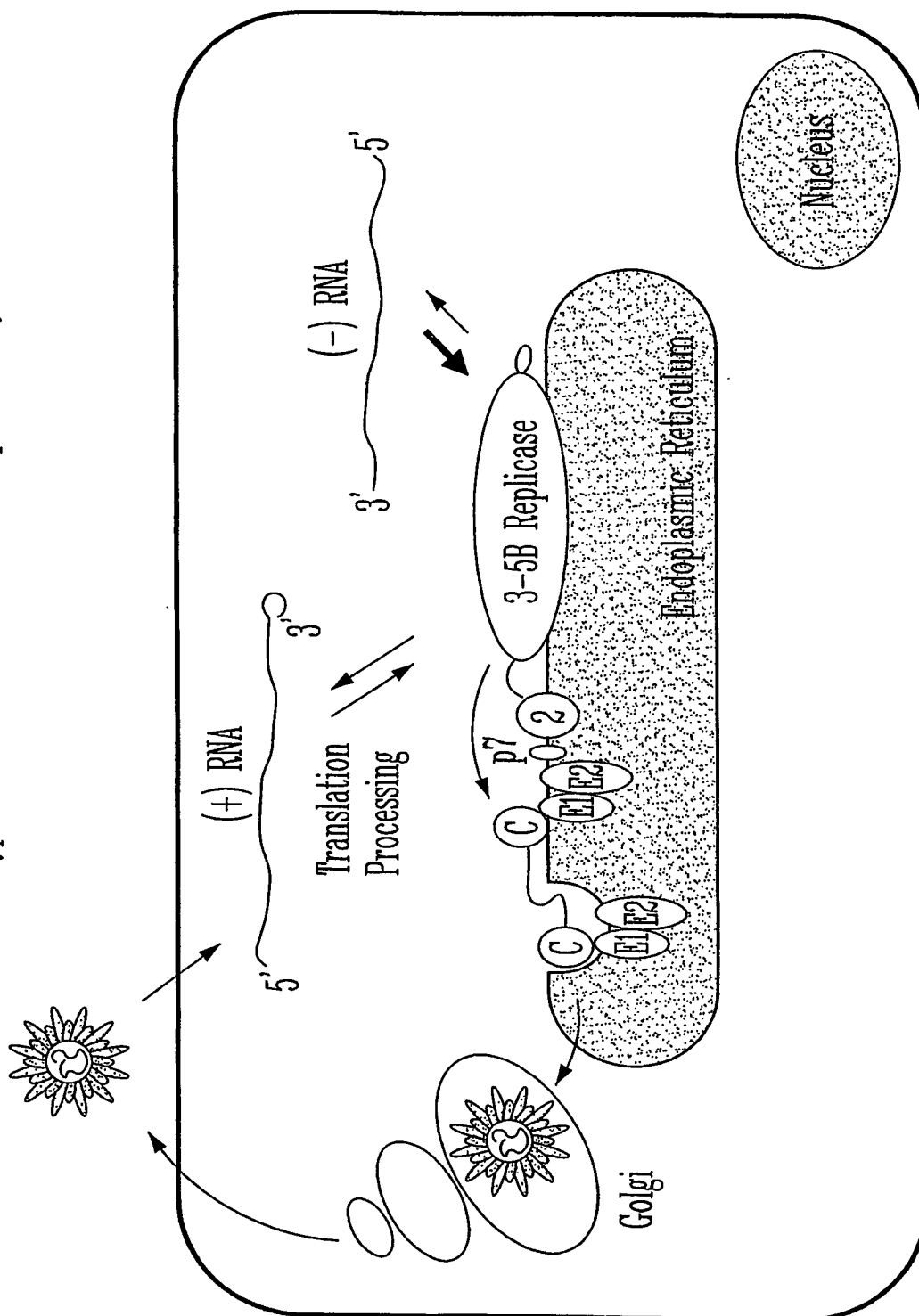


FIG. 2

**Experimental Protocol.**

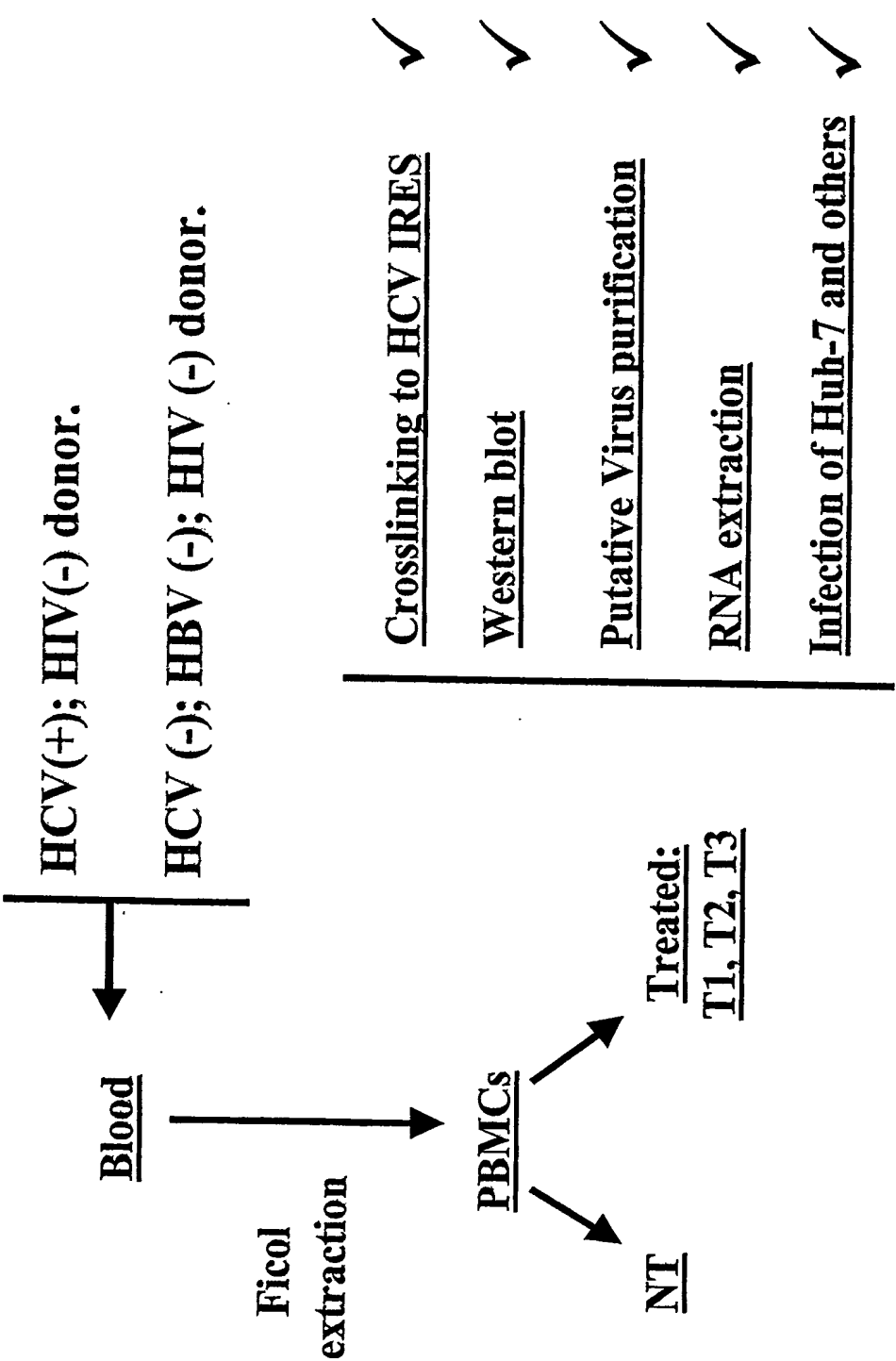
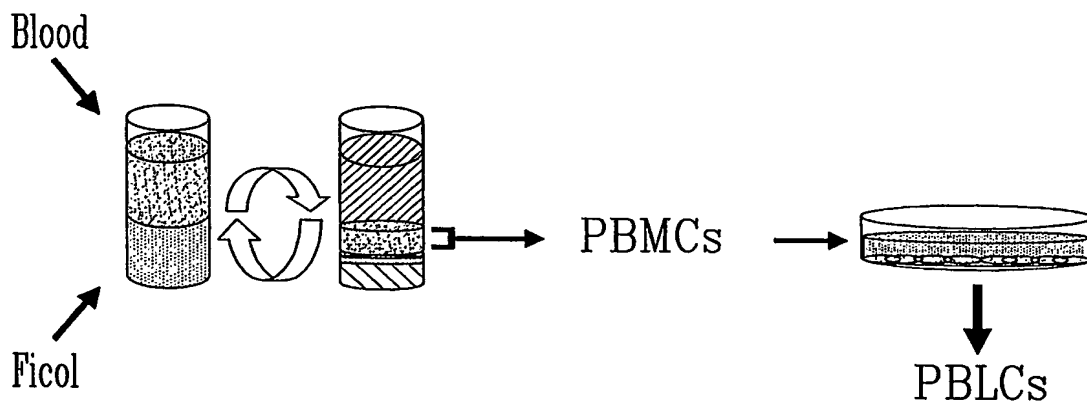


FIG. 3

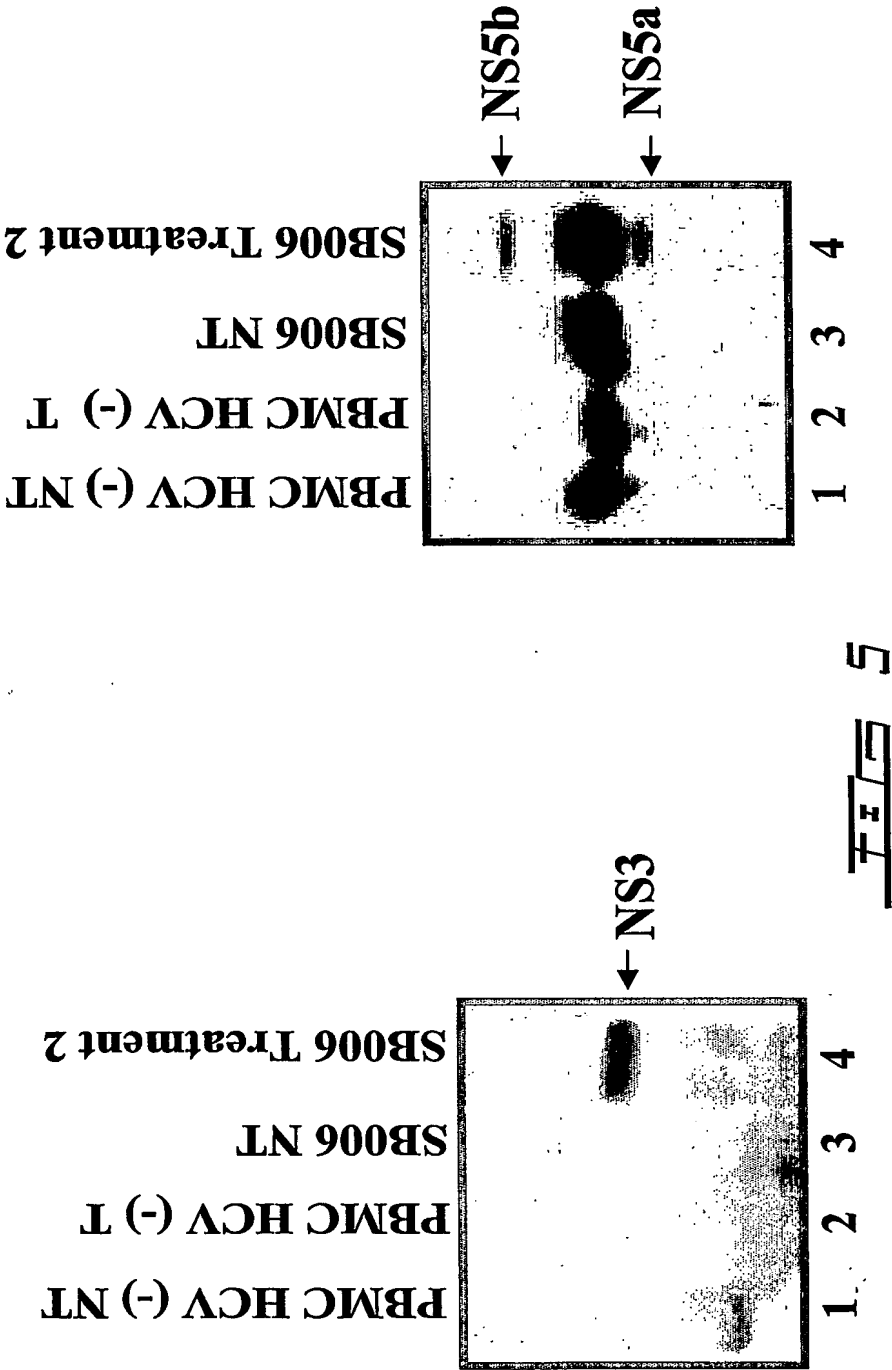
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PBMC and PBLC purification from blood samples.

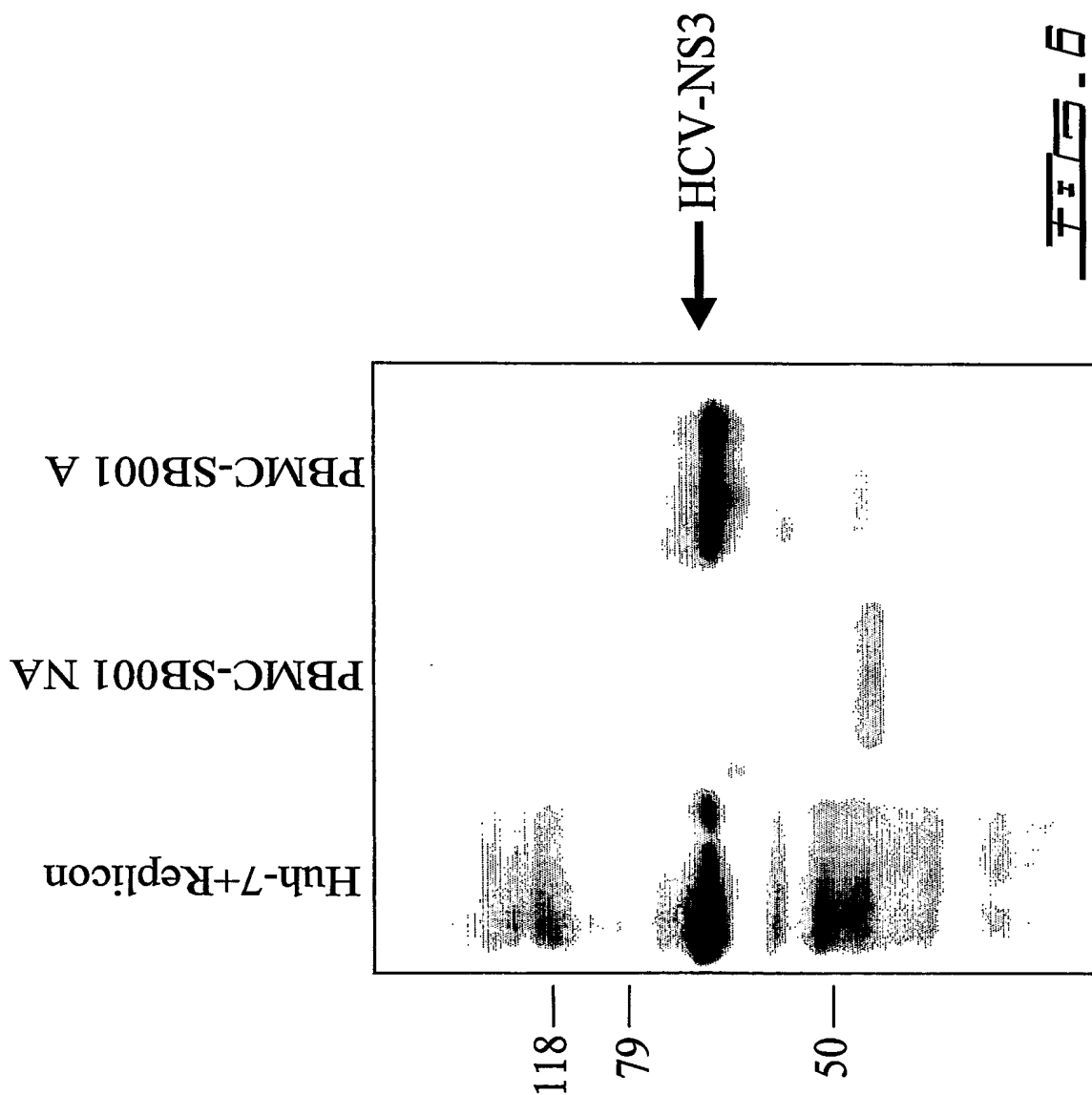
FIG. 4

Detection of HCV NS3 and NS5 proteins in cell extracts from Treated

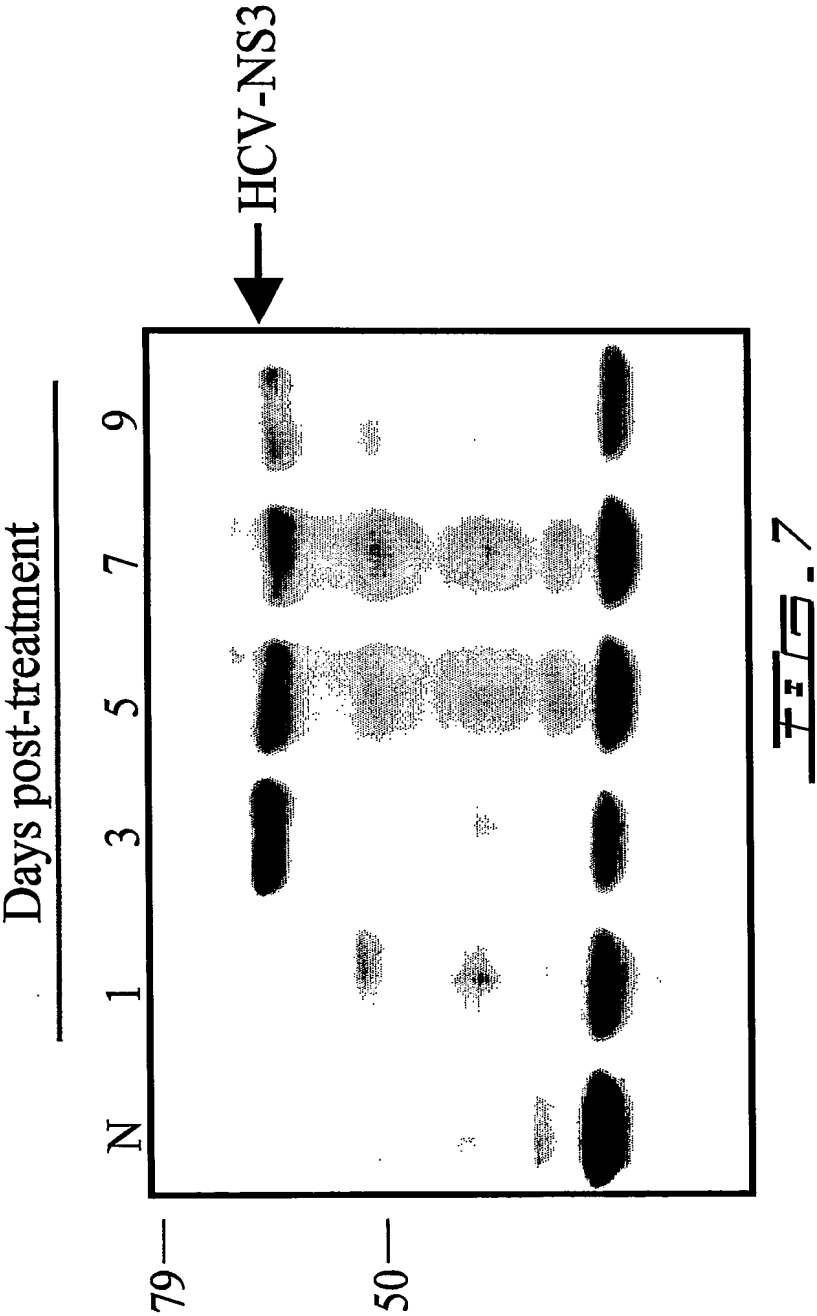
PBMC from an HCV (+) patient.  
[Boeringeranti-NS3 polyclonal antibody]



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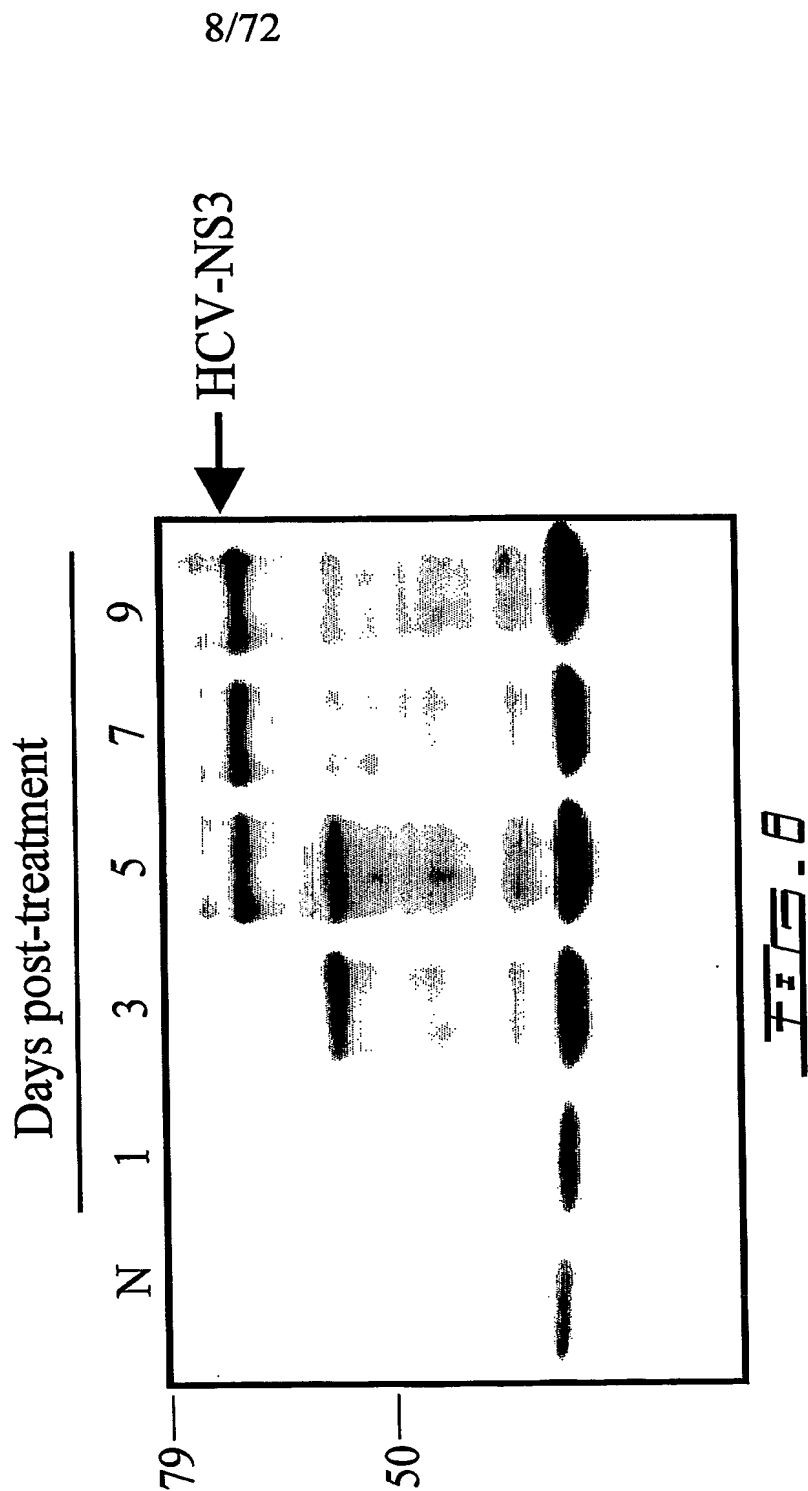


**Time course of HCV-NS3 detection:  
PBMCs From patient MLL-001**



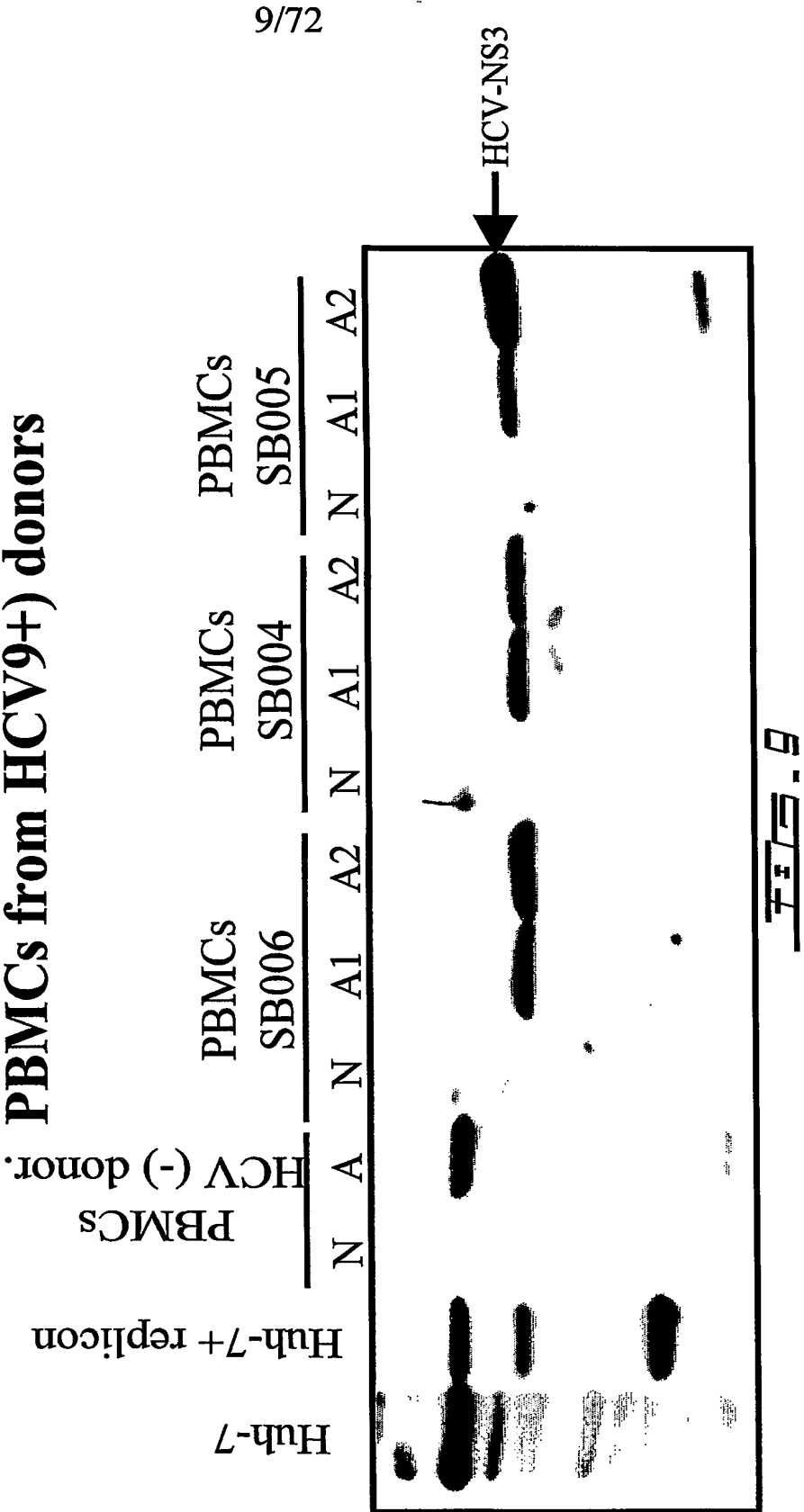
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# Time course HCV-NS3 detection: PBMCs from patient MLL-002

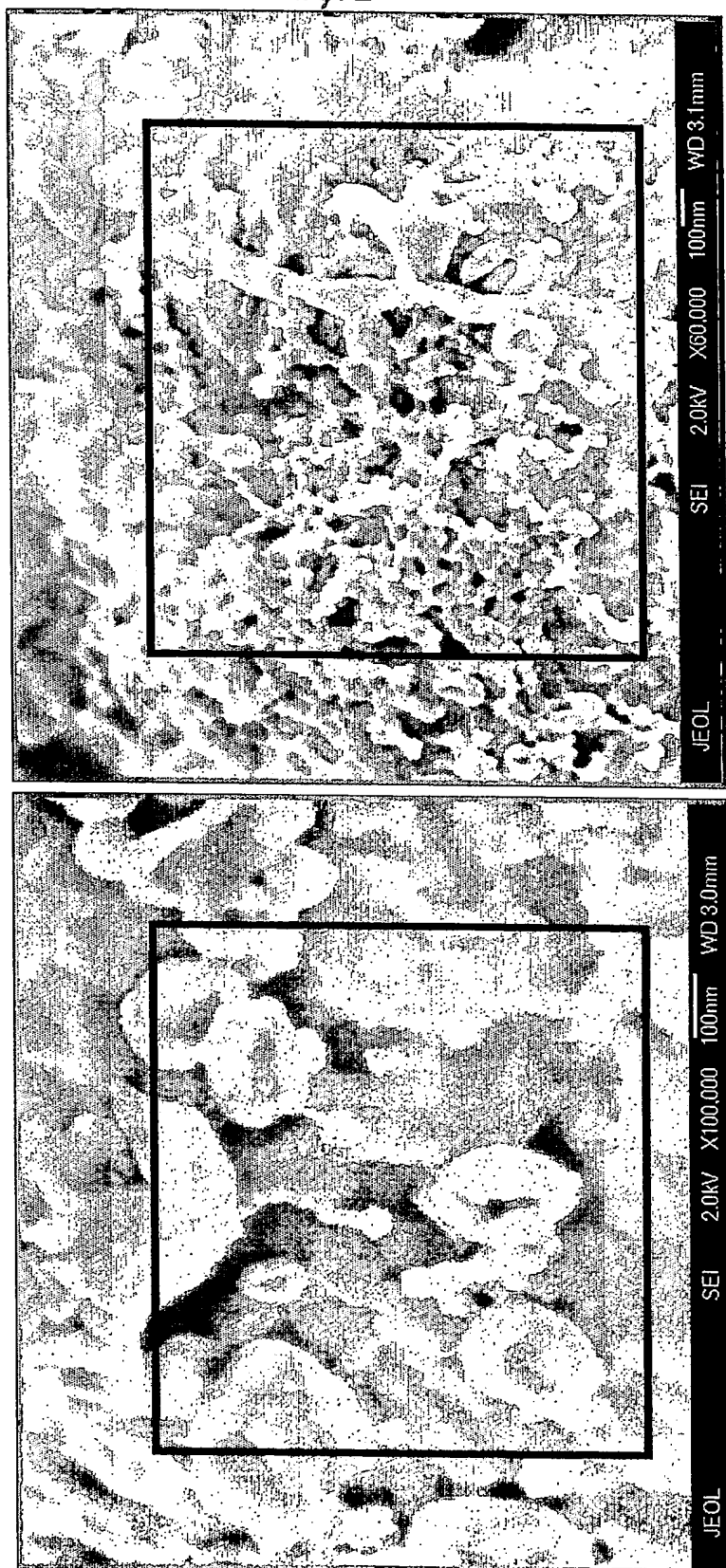




Detection of HCV-NS3 protein in treated (N3)  
PBMCs from HCV9+) donors



Detection of virus like particles by scanning electron microscopy

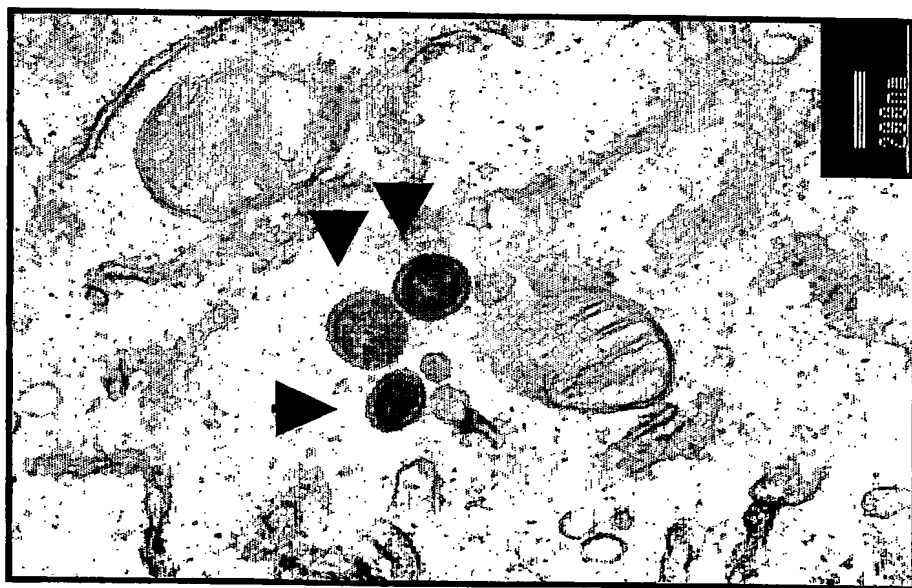


(-) Control

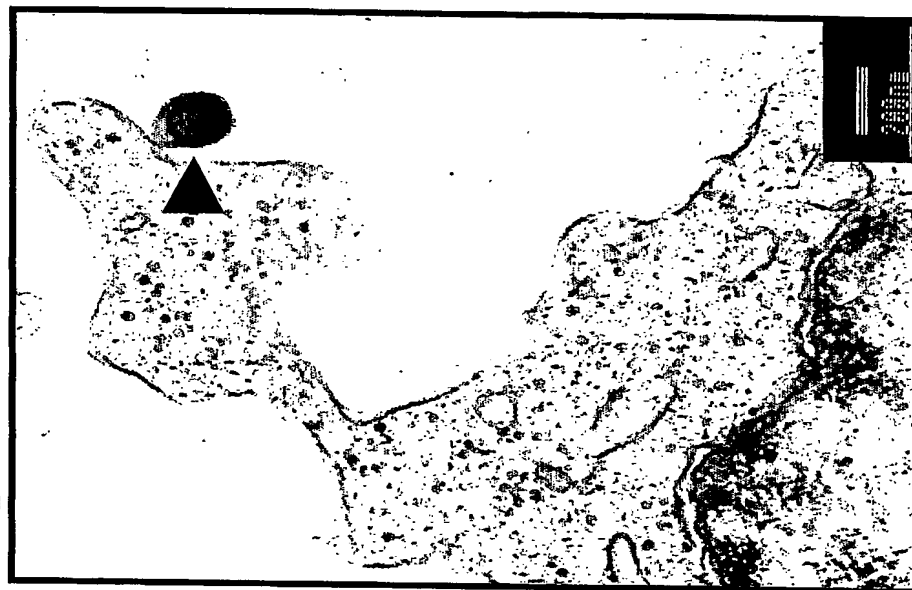
FIG. 10

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**Electron microscopy of Activated PBLCs;  
Detection of virus like particles**



200 nm



200 nm

FIG. 11

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Virus partial purification.

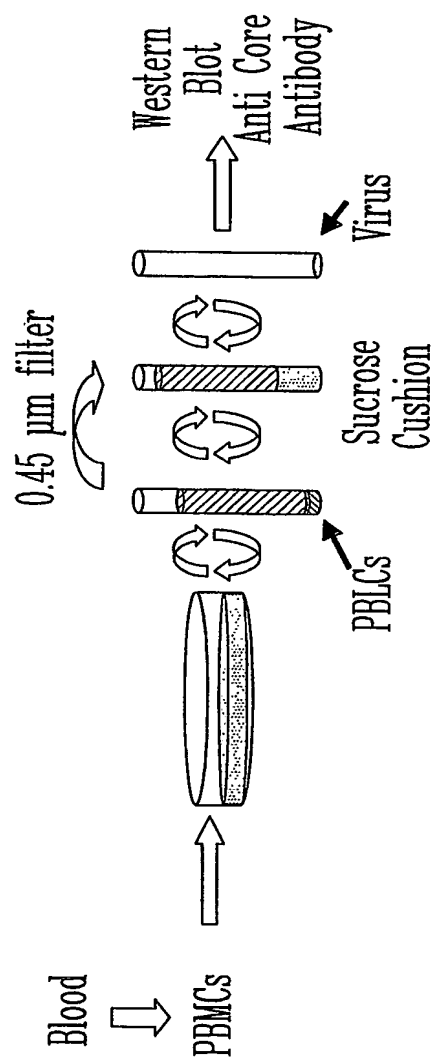
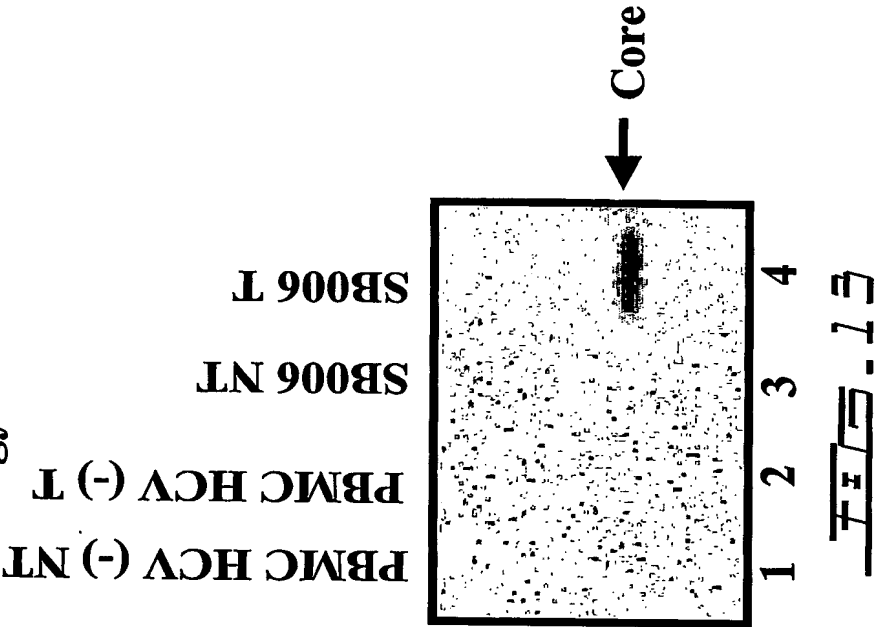


FIG. 12

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Detection of HCV Core protein in supernatant of treated  
PBMC from an HCV (+) patient.  
[Maine biotechnology anti-Core monoclonal antibody]

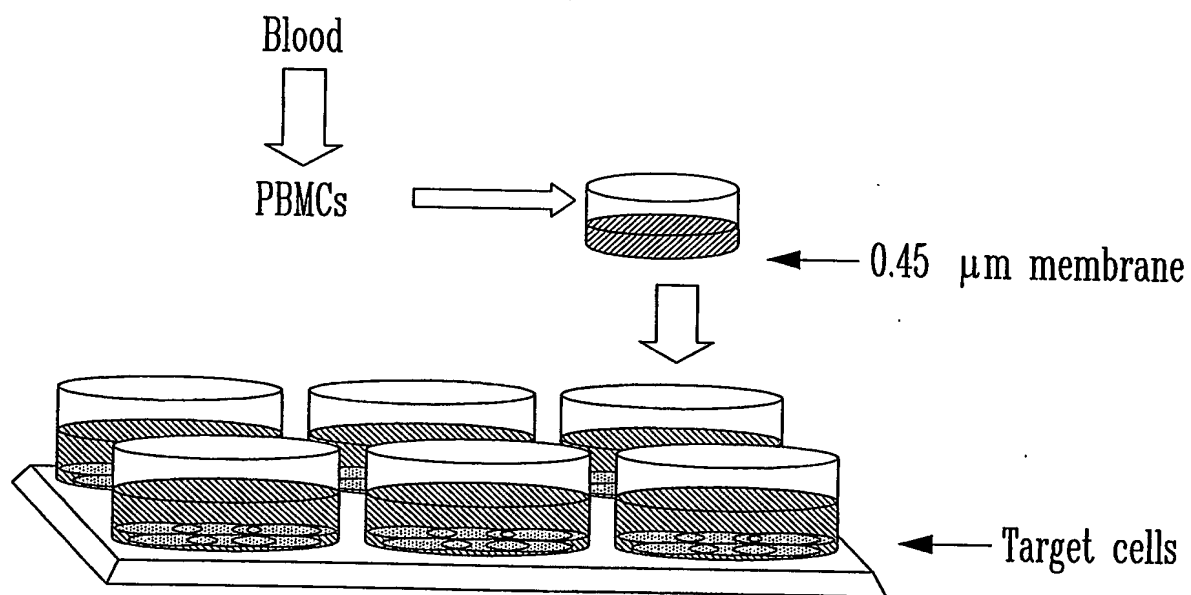


RNA Quantification I (virus copies/ng total RNA)

Patient	HCV RNA In PBMC	Detection of Core (wb) in supernatant
<u>After 4 days</u>		
SB004 NT	2x10 <sup>3</sup>	No
SB004 T	2x10 <sup>3</sup>	Yes
SB006 NT	1.8 x10 <sup>3</sup>	No
SB006 T	2x10 <sup>2</sup>	Yes
<u>After 20 days</u>		
SB004	0.00	
SB006	0.00	<u>7x10<sup>3</sup></u> - 14

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## Infection assay; co-culture

FIG. 15

**Infection of MT-4 cells**  
**RNA Quantification II (virus copies/ng total RNA)**

<b>Patient</b>	<b>HCV RNA In PBMC</b>	<b>Detection of Core (wb) in supernatant</b>	<b>HCV RNA In MT-4</b>
<u><b>After 10 days</b></u>			
<b>SB001 NT</b>	<b>13</b>	<b>No</b>	<b>0.00</b>
<b>SB001 T</b>	<b>12</b>	<b>Yes</b>	<b>1600</b>
<u><b>After 20 days</b></u>			
<b>SB001</b>	<b>0.00</b>		<b>0.00</b>
<b>SB001</b>	<b>0.00</b>		<b>0.00</b>

**FIG. 16**



## Co-culture of Huh-7 and HCV (-) PBMCs.

- 1- Huh-7
- 2- Huh-7 + PBMCs HCV (-) NT
- 3- Huh-7 + Treatment
- 4- Huh-7 + PBMCs HCV (-) T

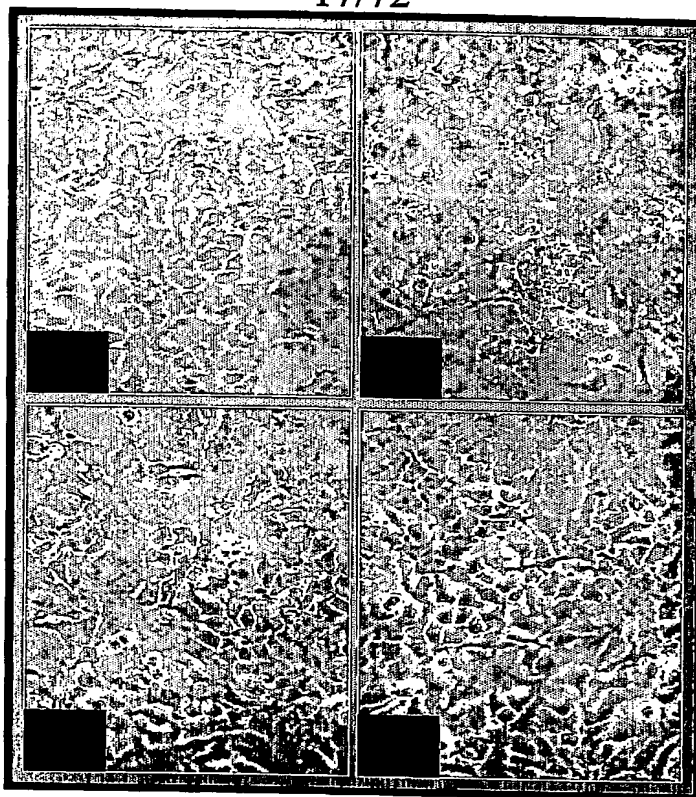
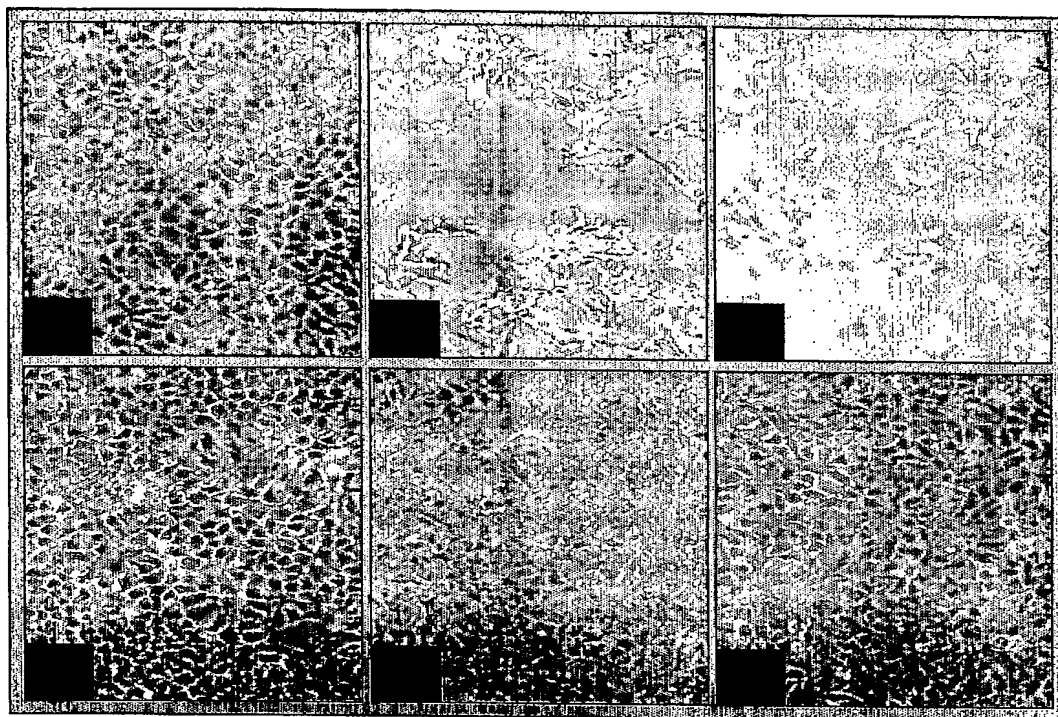


FIG. 17

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# Co-culture of Huh-7 and HCV (+) PBMS° Cs (SB006).

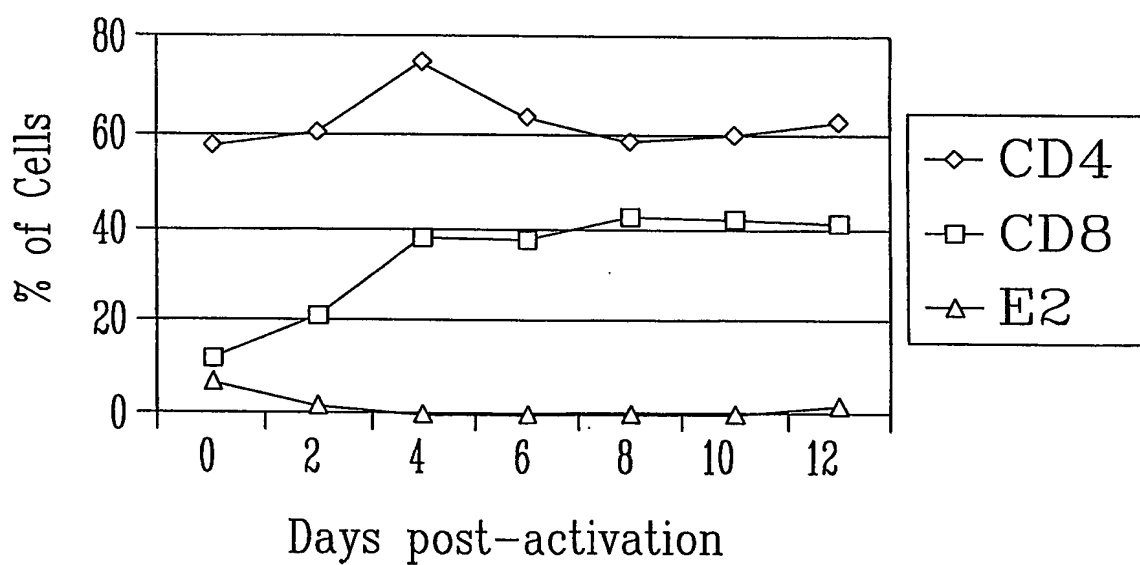


1. Huh-7
- 2-3. Huh-7 + PBMCs HCV (+) NT
4. Huh-7 + Treatment
- 5-6. Huh-7 + PBMCs HCV (+) T

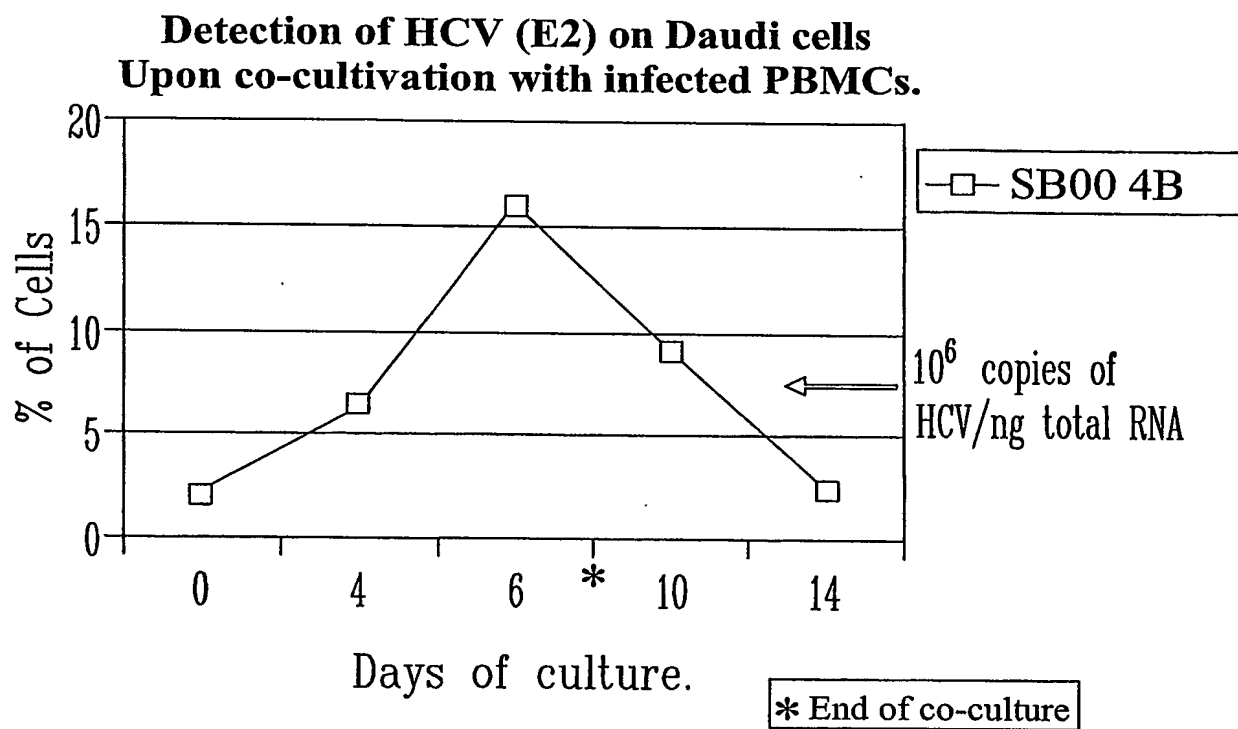
FIG. 18

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PHA Activation of PBMCs from patient SB004;  
HCV is not in T cells

FIG - 19

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FIG. 20

Comparison of different activation treatments;  
PBMCs from donor MLL-010

		T+B cells				Treatment			
		T cells (T1)				B cells (T2)			
		(T3)				(T3)			
N	2	4	8	12	2	4	8	12	4
		Days				Days			

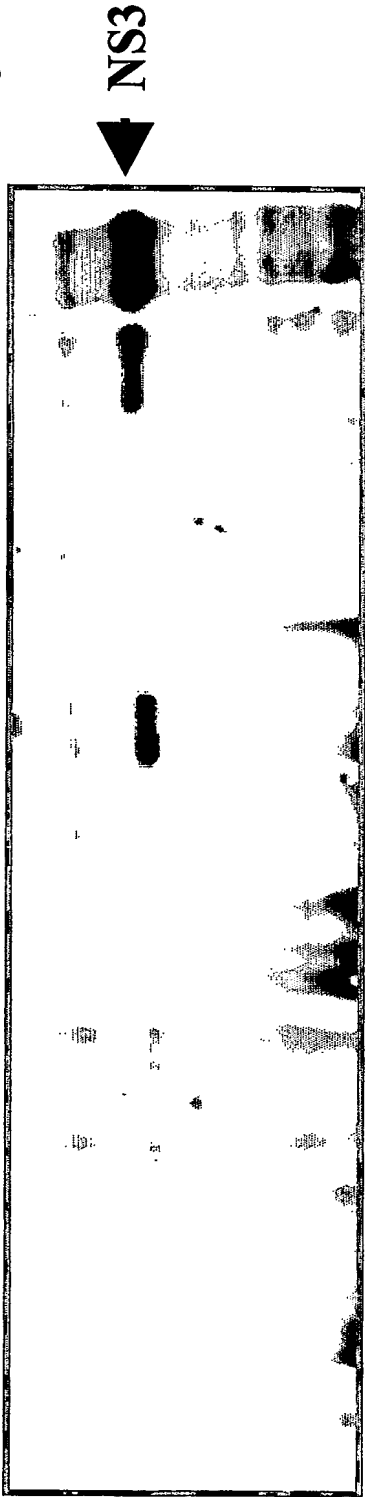


FIG. 21

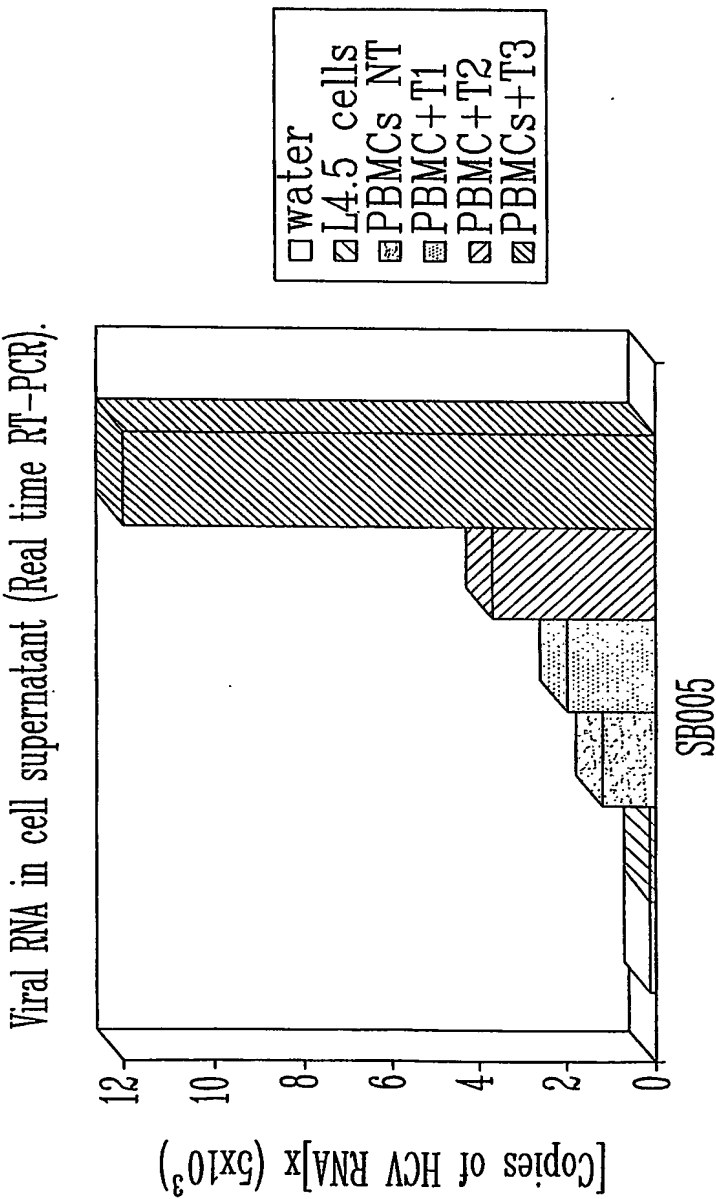


Fig. 22

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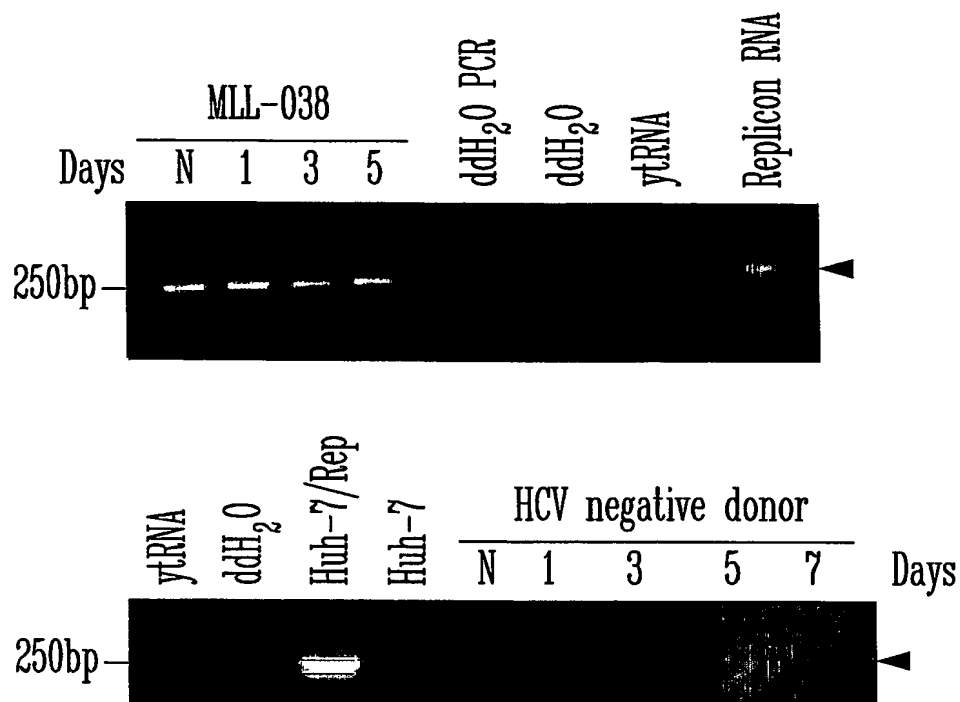


FIG. 23 A

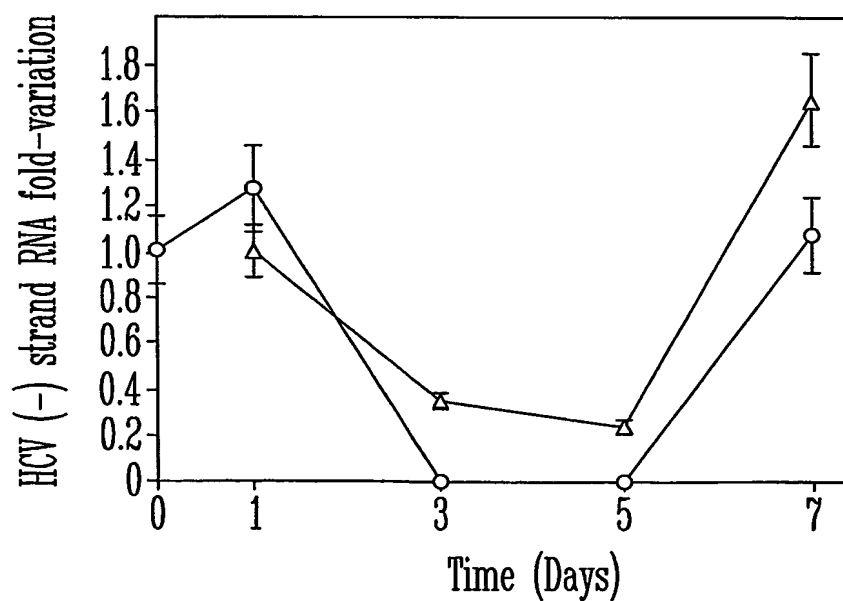


FIG. 23 B

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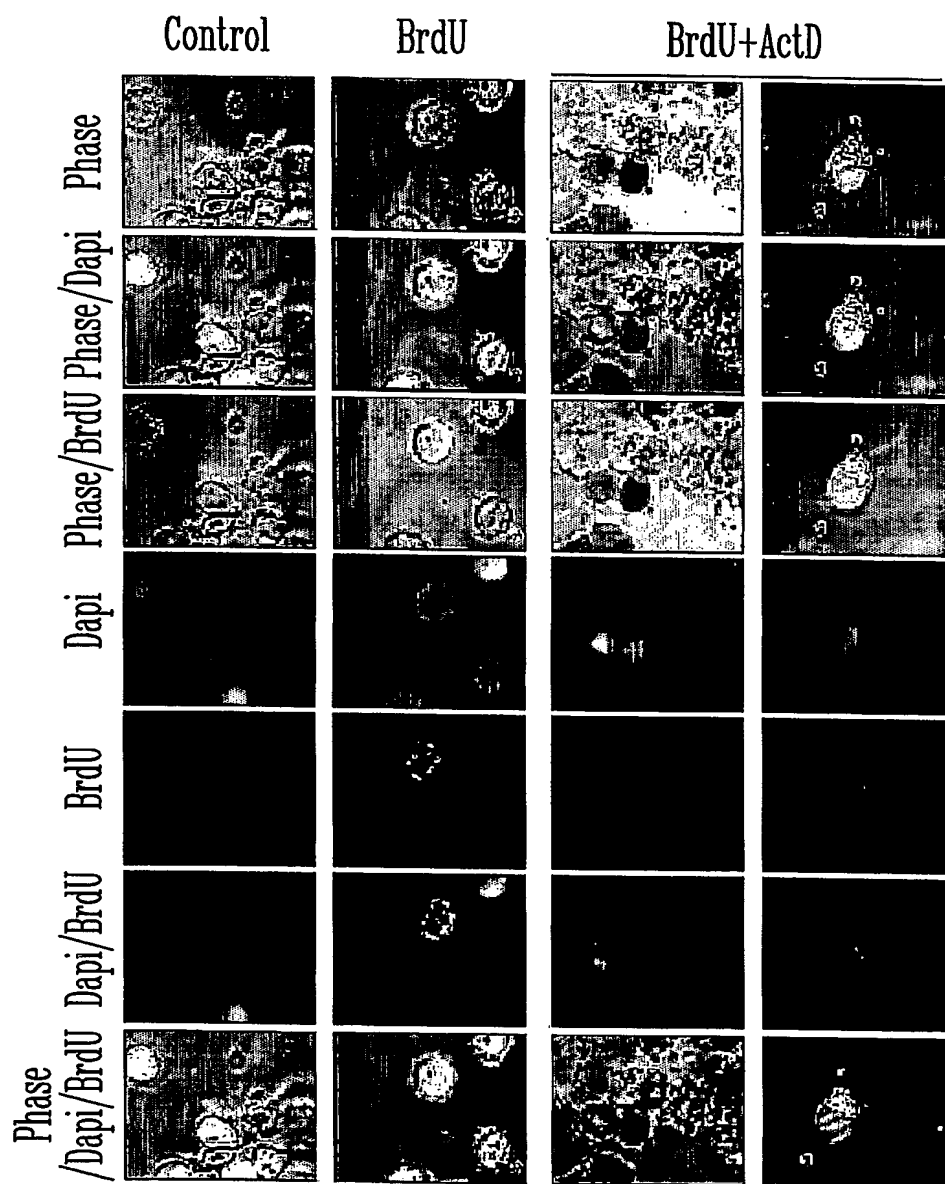


FIG. 23C



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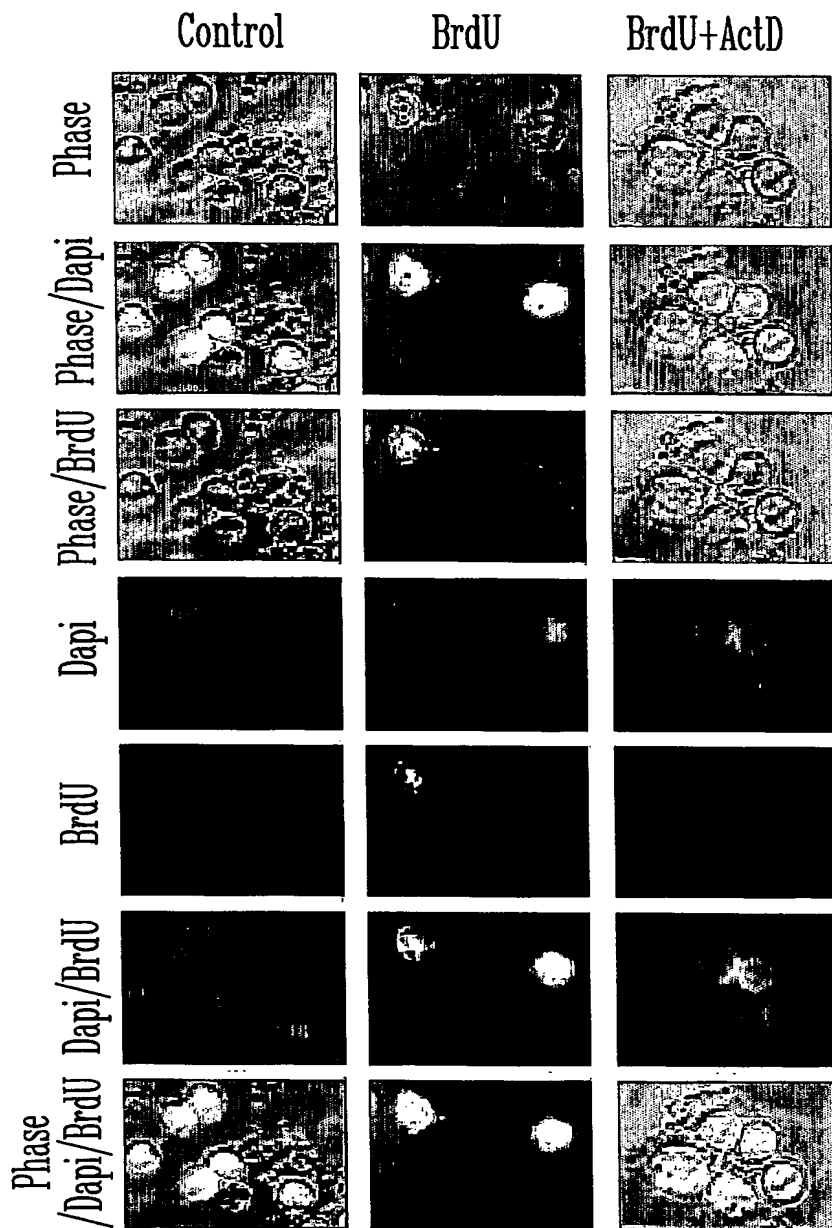
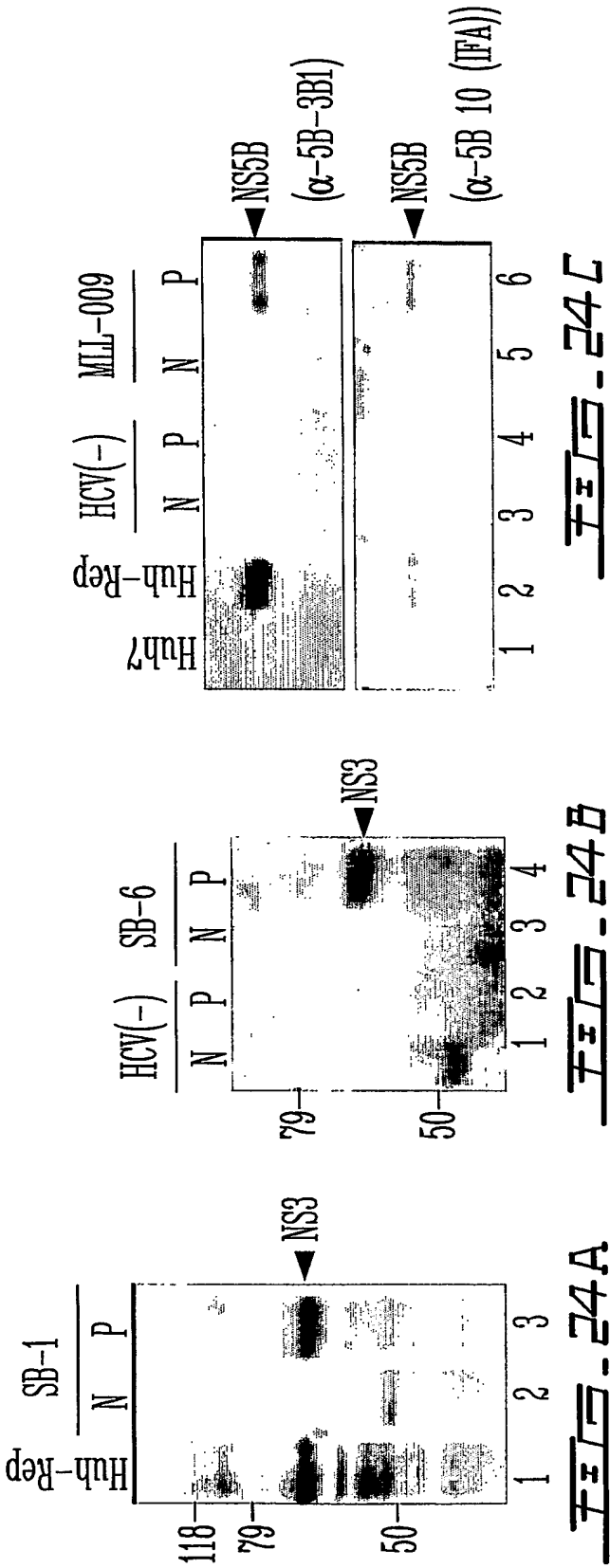


FIG. 23D



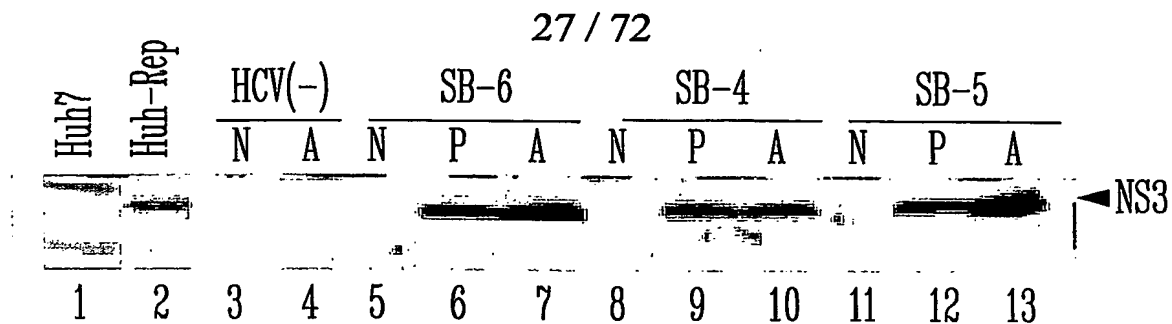


FIG. 24 D

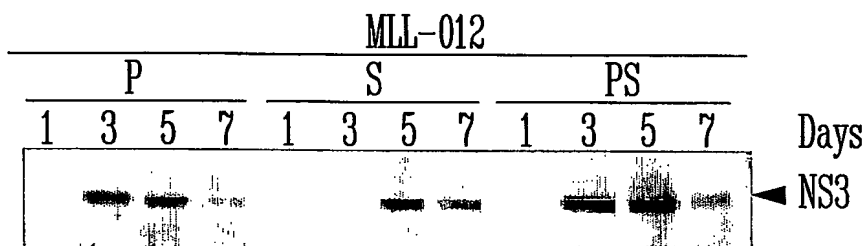


FIG. 24 E

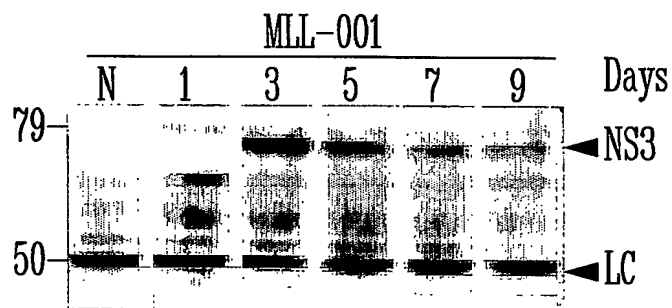


FIG. 24 F

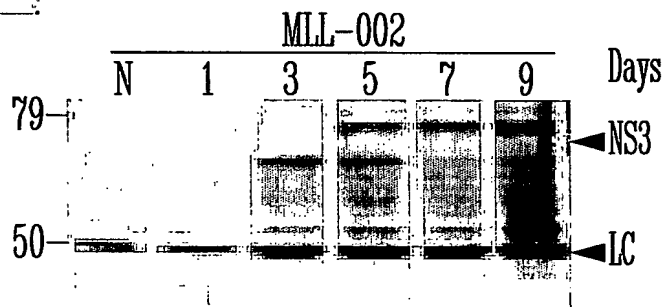


FIG. 24 G

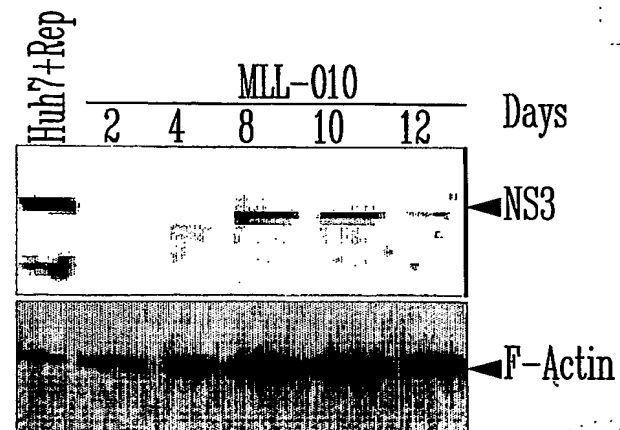


FIG. 24 H

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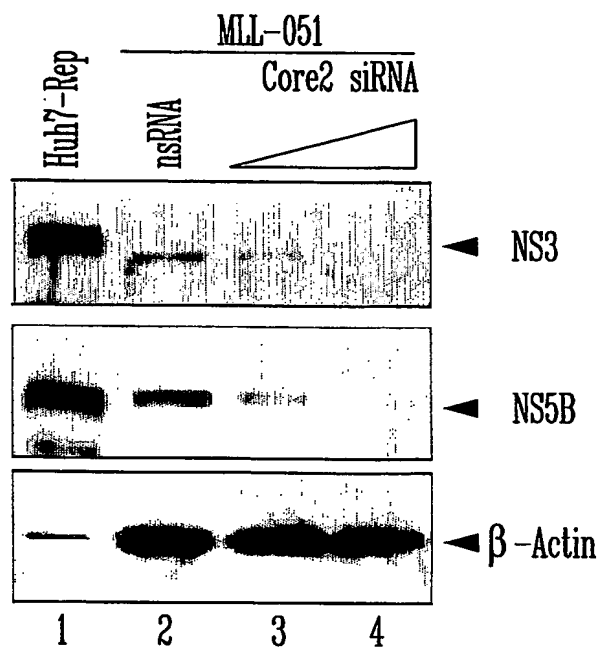


FIG. 24I

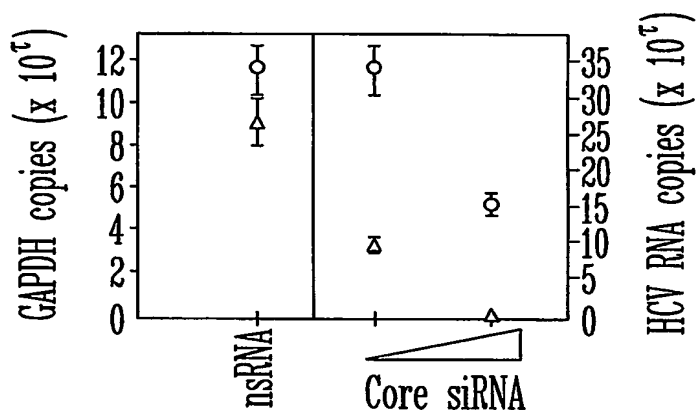
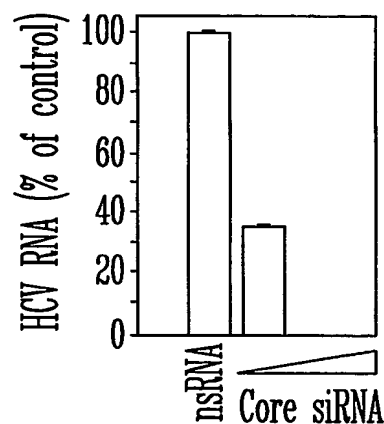


FIG. 24J

FIG. 24K



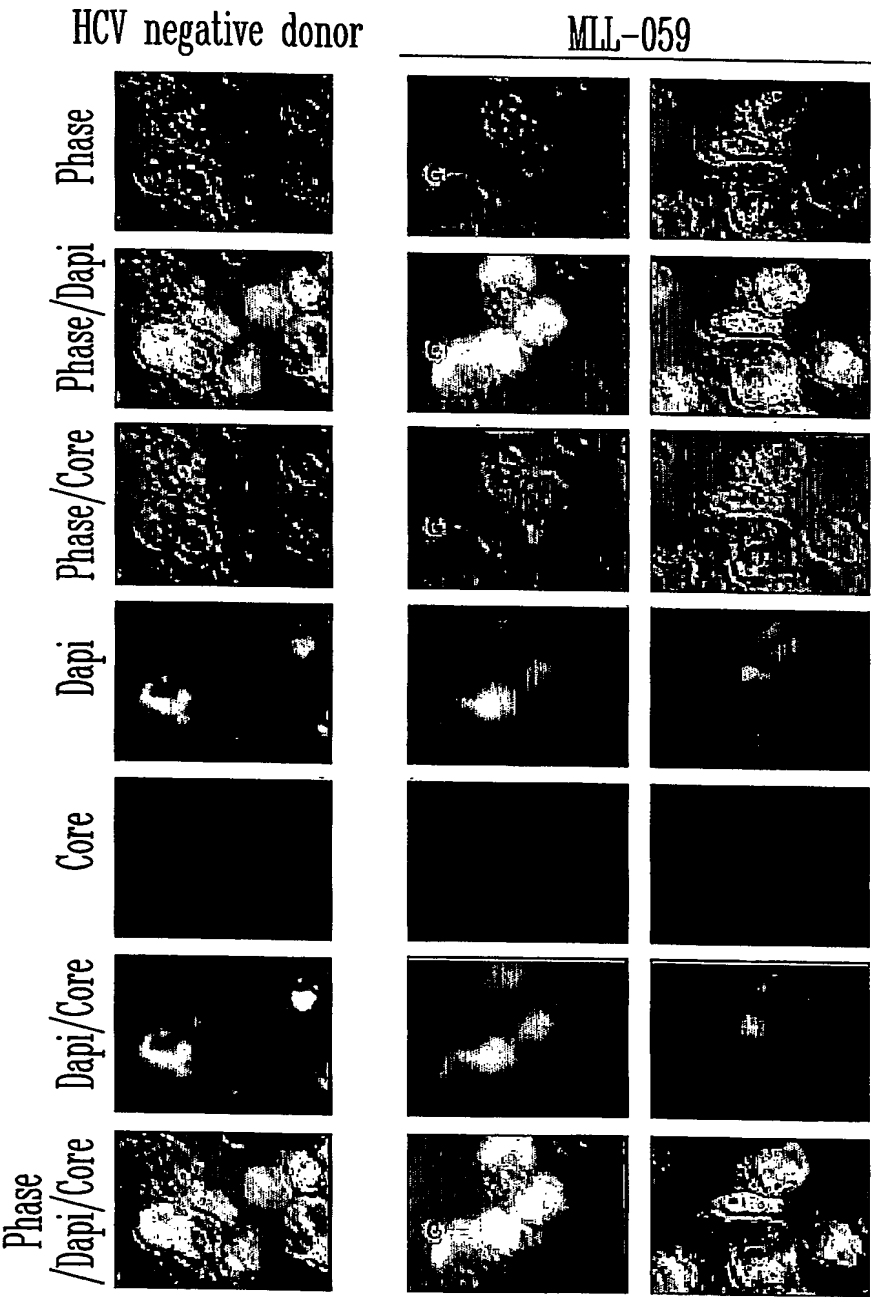


FIG. 25

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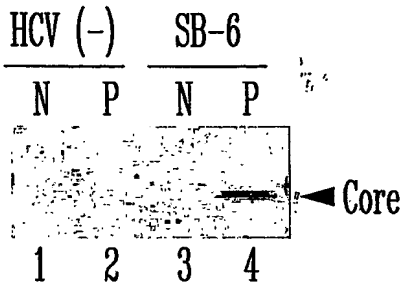


FIG. 26A

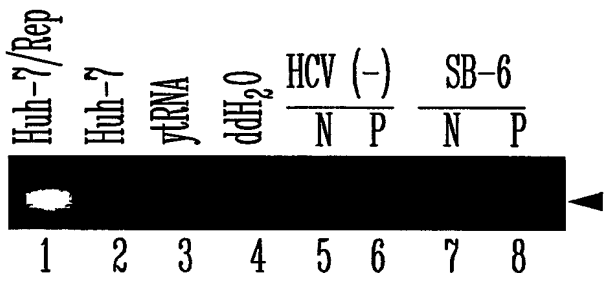
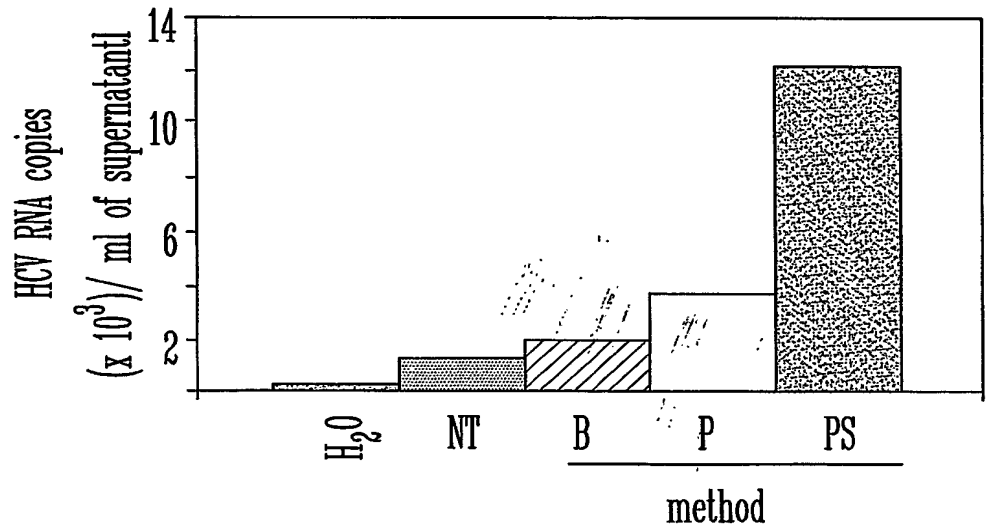


FIG. 26B



SB-5  
FIG. 26C

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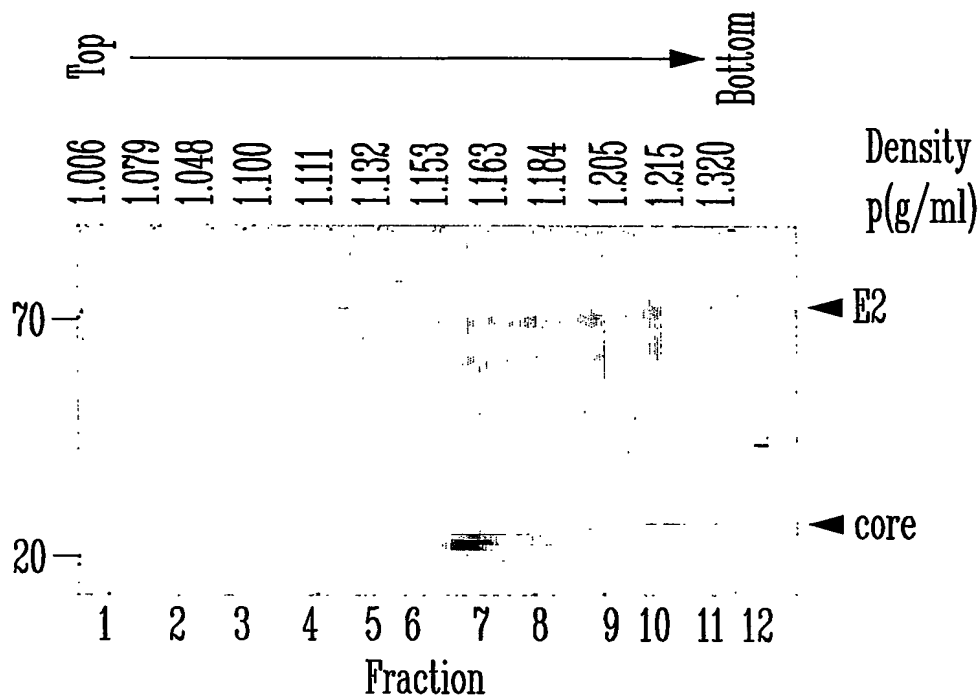


FIG. 260

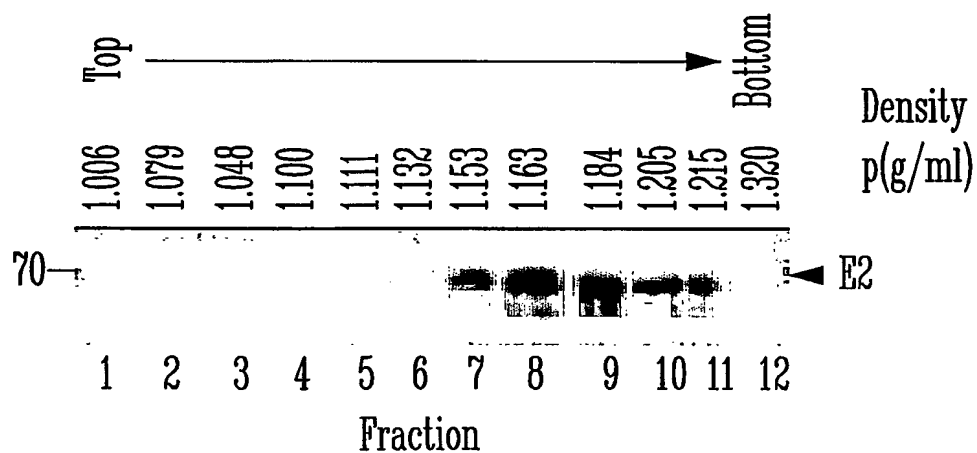


FIG. 26E

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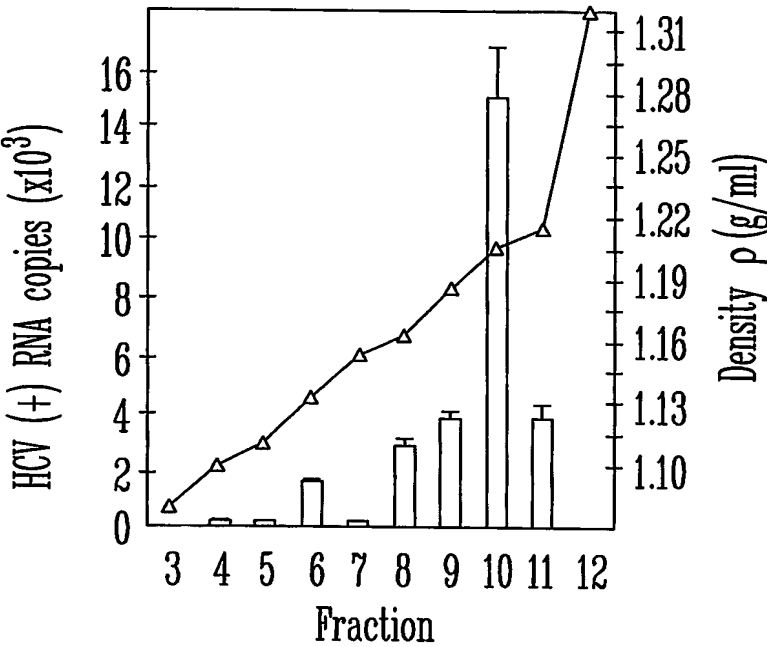


FIG. 26F

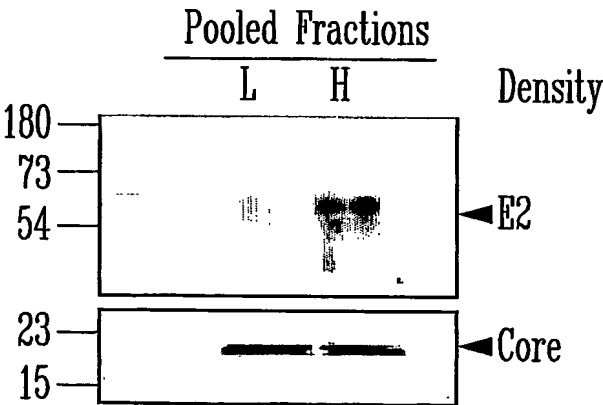


FIG. 26G



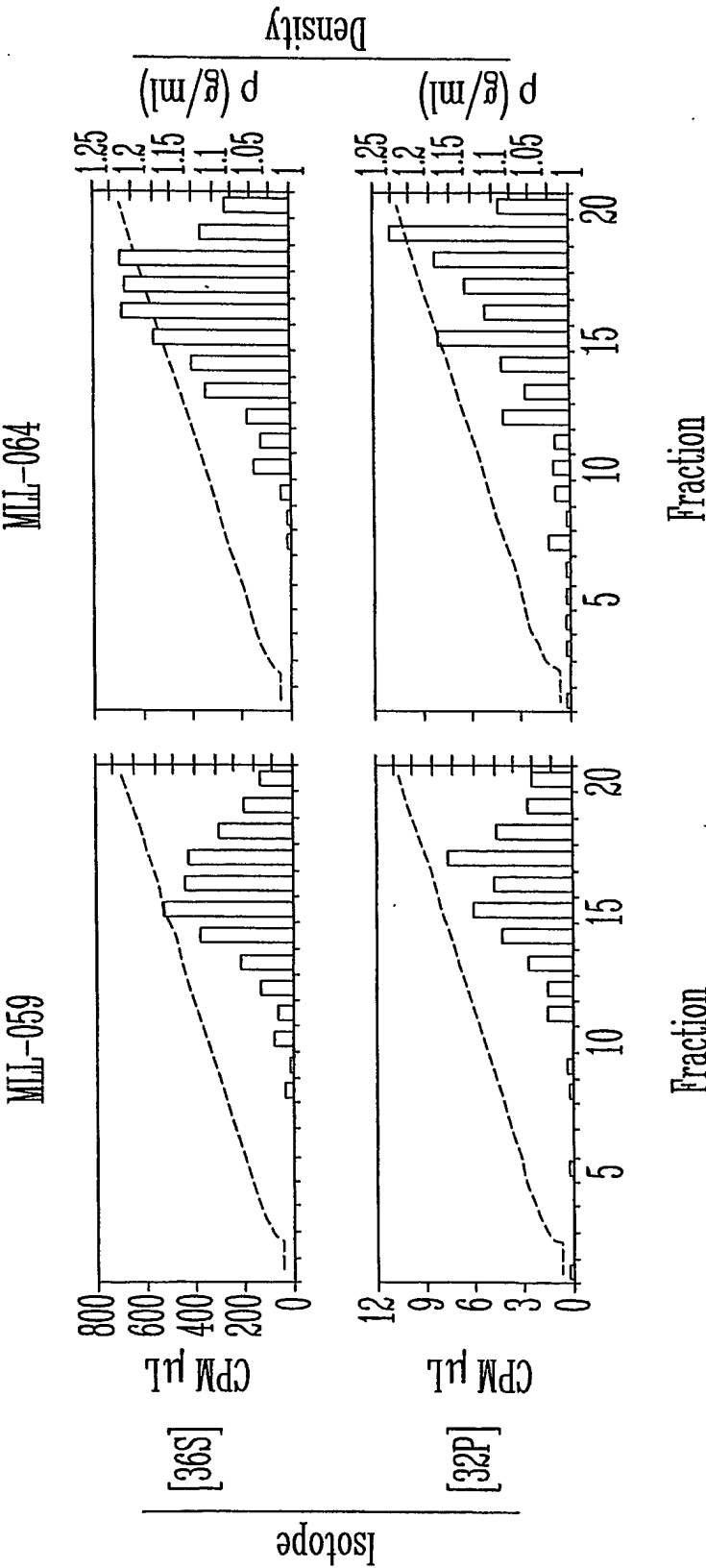


FIG. 26H

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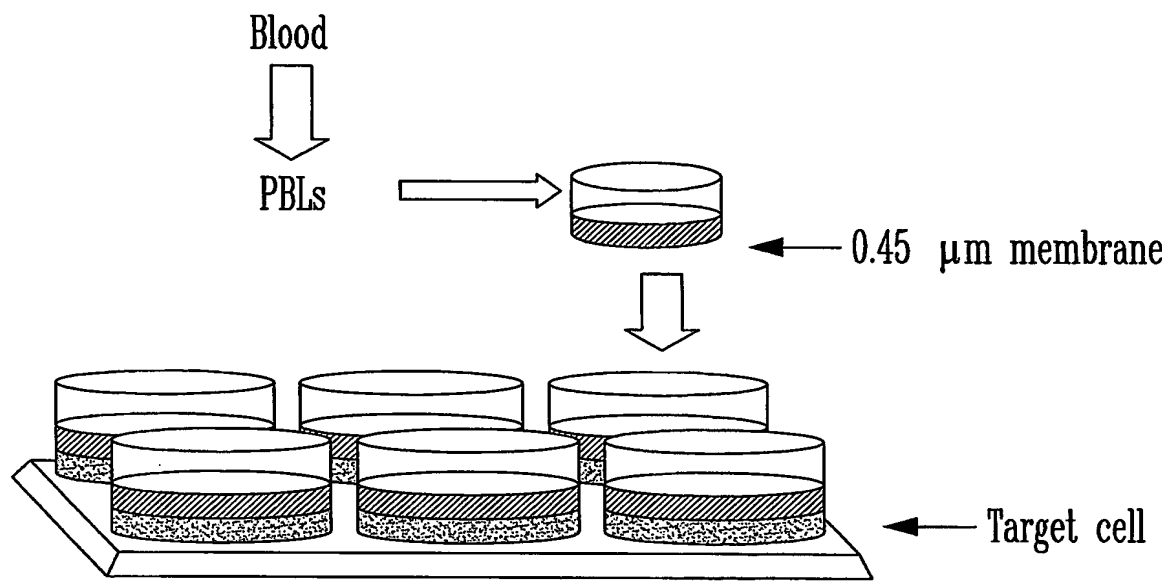


FIG. 27A

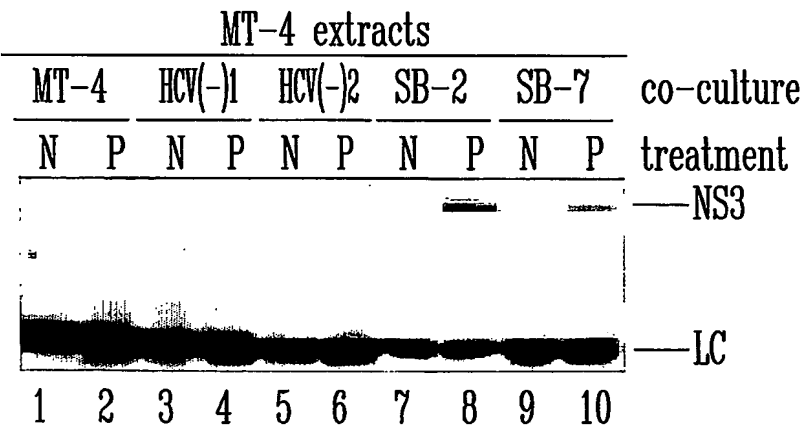


FIG. 27B

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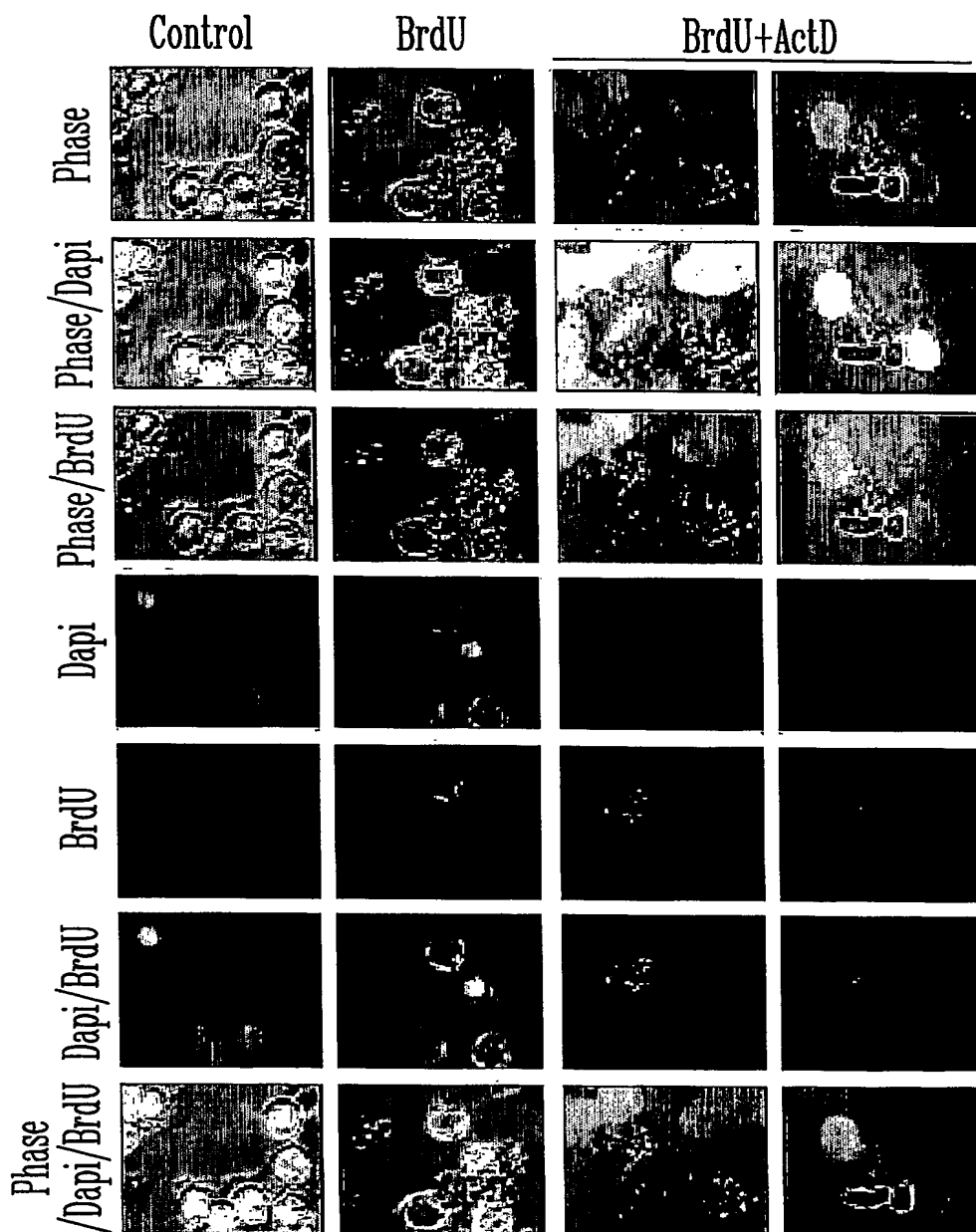


FIG. 2B

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# HCV Replication Cycle

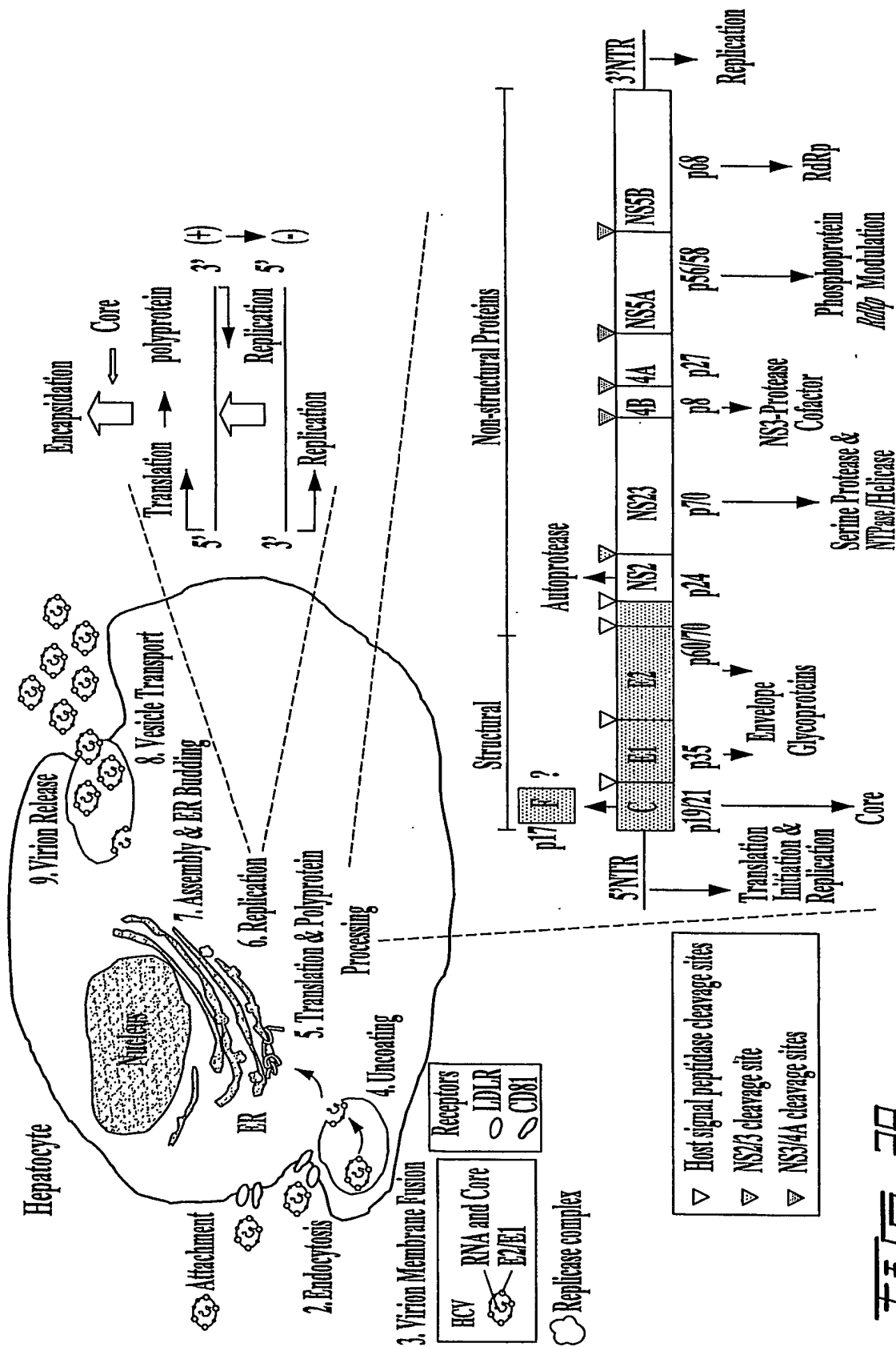
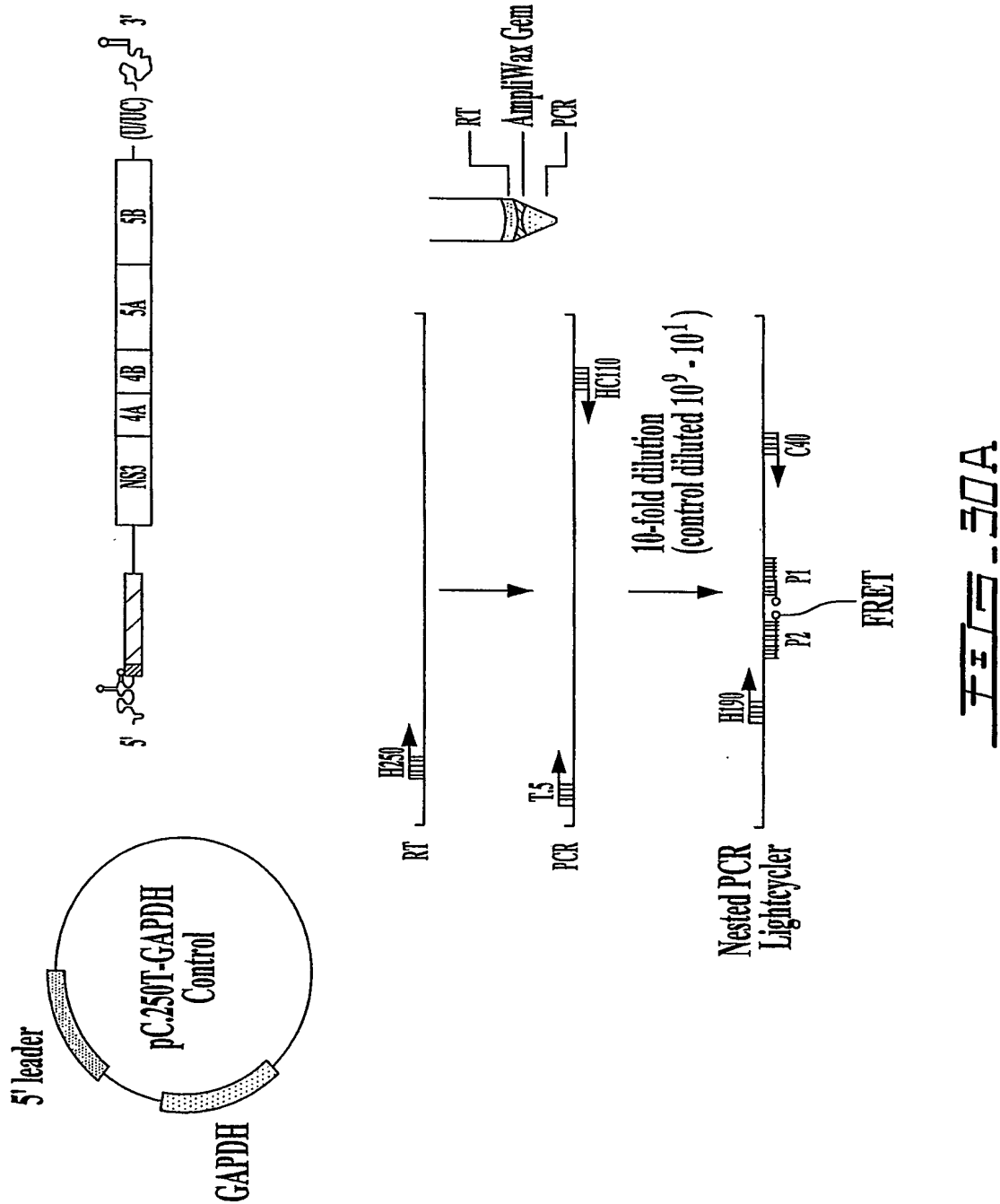
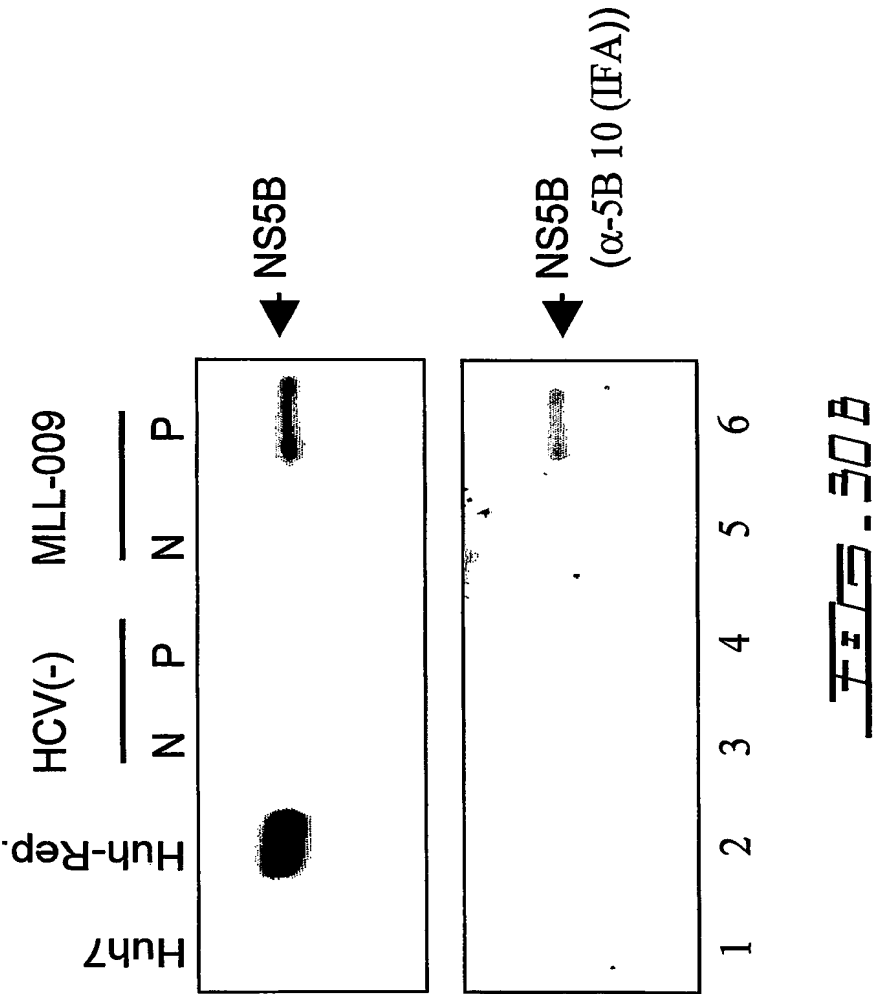


FIG. 29



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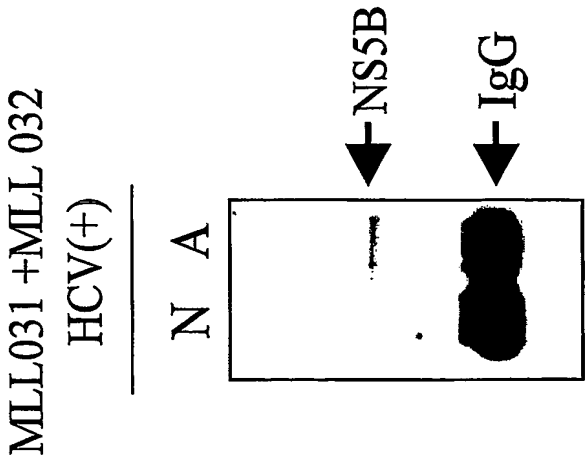
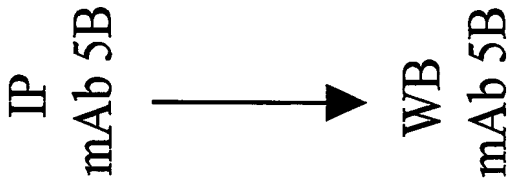
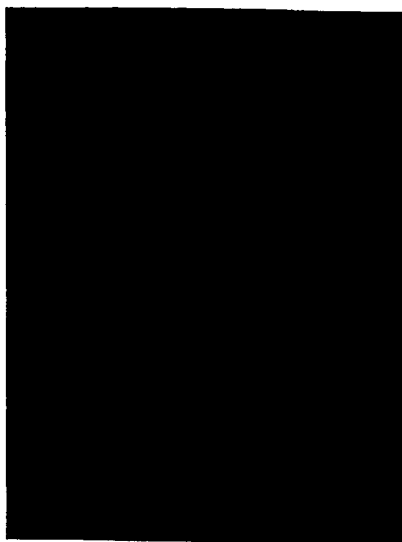


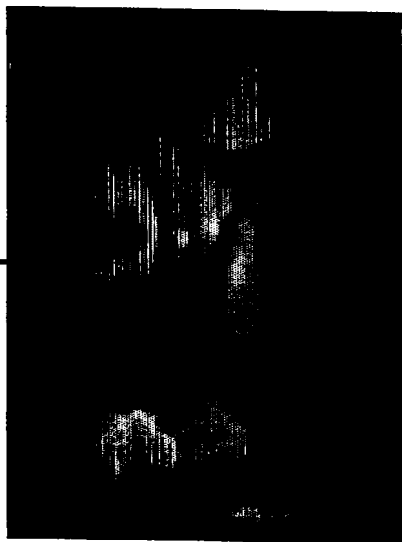
FIG. 31

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Anti-Core



Dapi



Phase



Phase/Dapi/Anti-Core



Dapi/Anti-Core

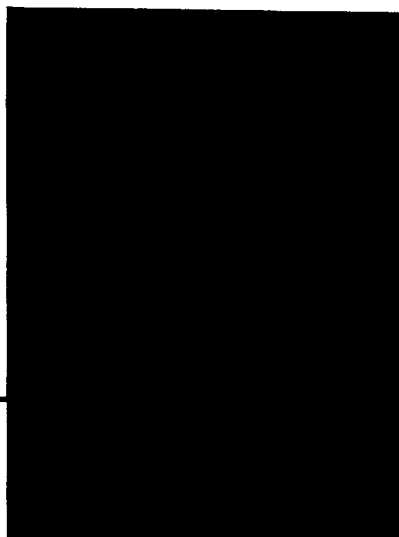


FIG. 32



BEST AVAILABLE COPY

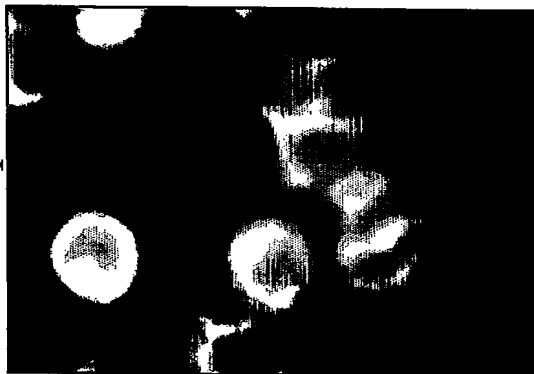
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FIG. 33

Anti-Core



Dapi



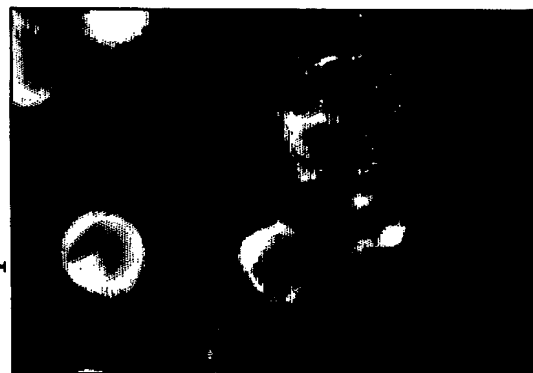
Phase



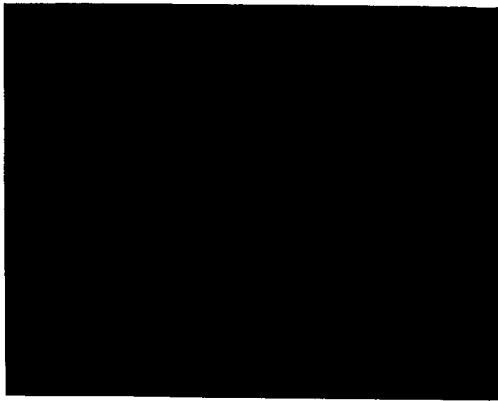
Phase/Dapi/Anti-Core



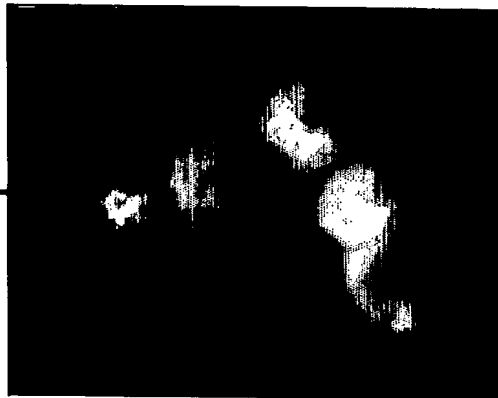
Dapi/Anti-Core



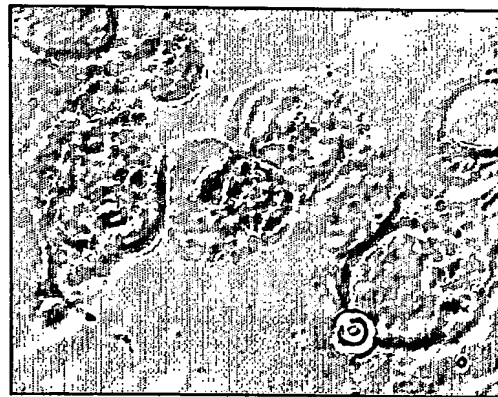
Anti-Core



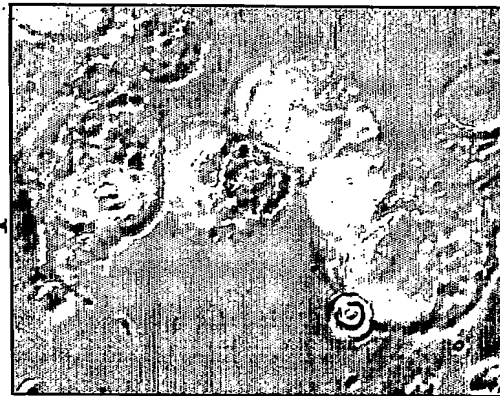
Dapi



Phase



Phase/Dapi/Anti-Core



Dapi/Anti-Core

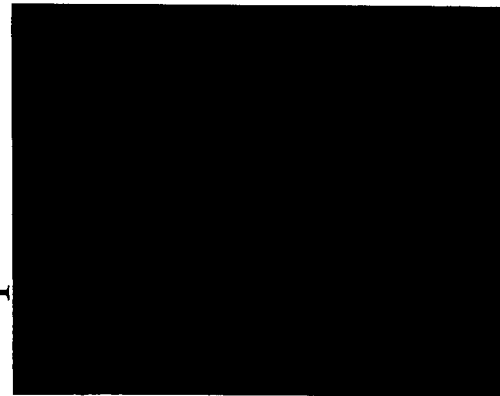
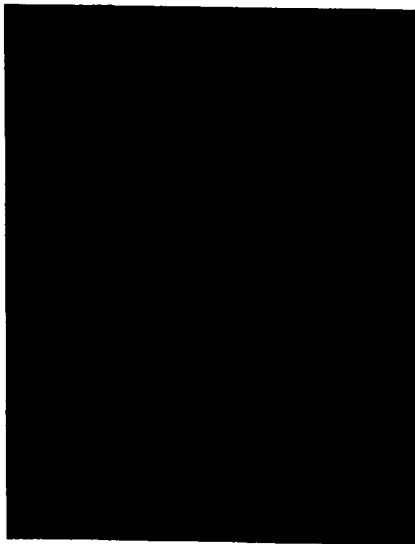


FIG. 34

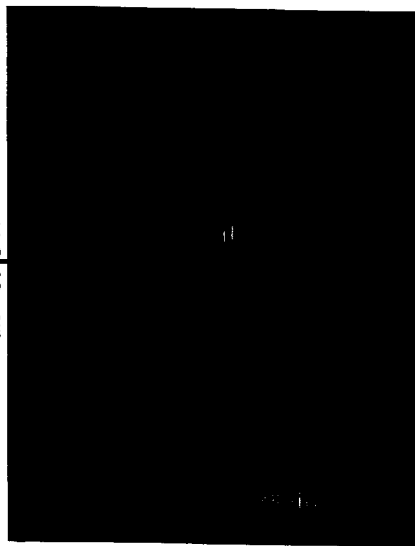
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Anti-Core



Dapi



Phase



Phase/Dapi/Anti-Core



Dapi/Anti-Core

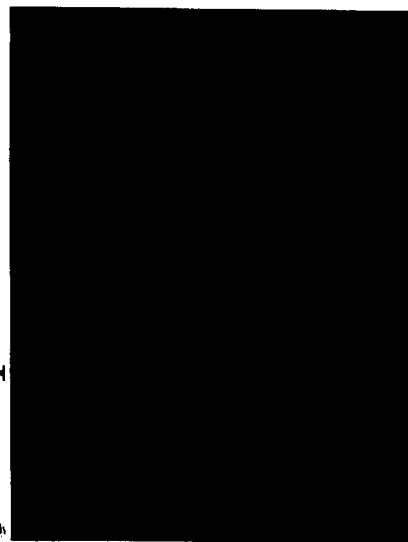
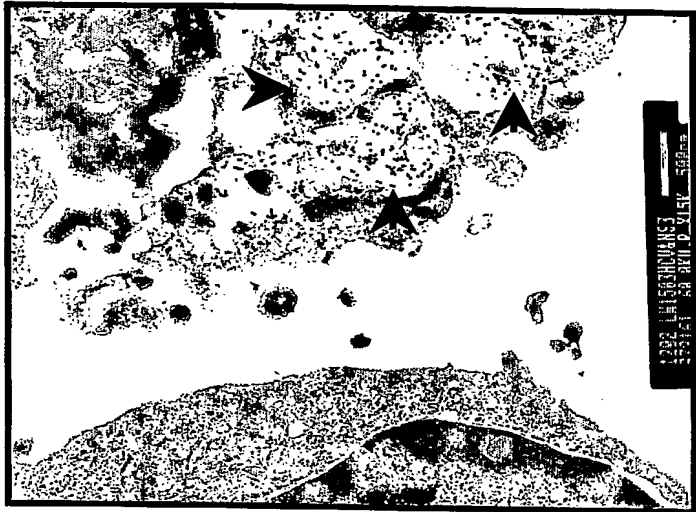


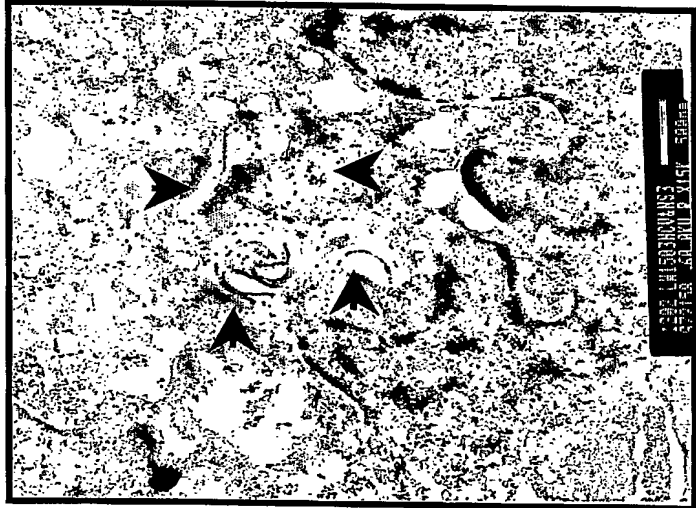
FIG. 35

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796-5134



796-5134



796-5134

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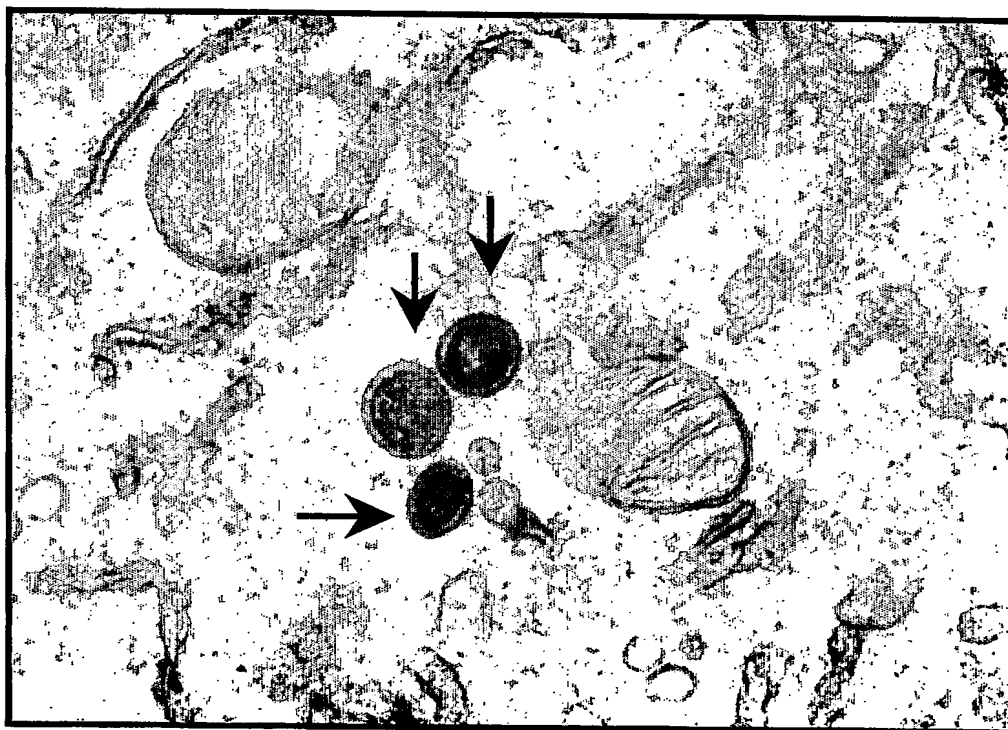
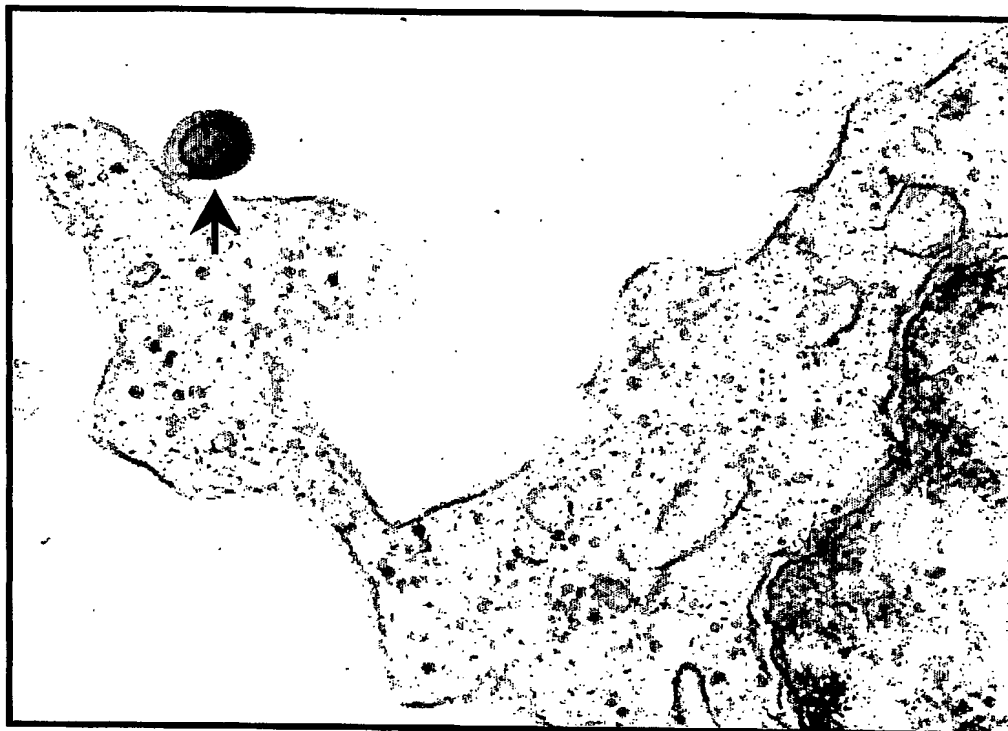


FIG. 37

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Virus partial purification.

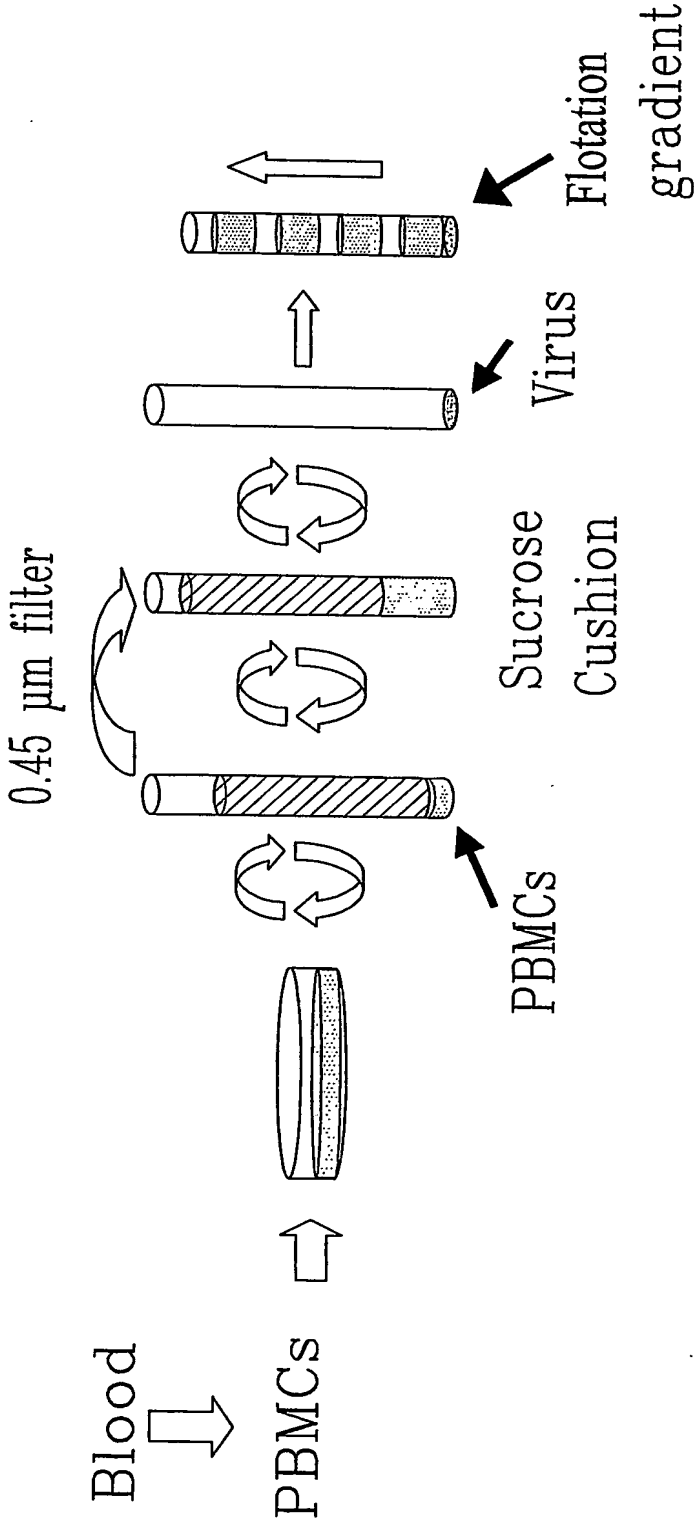


FIG. 3B

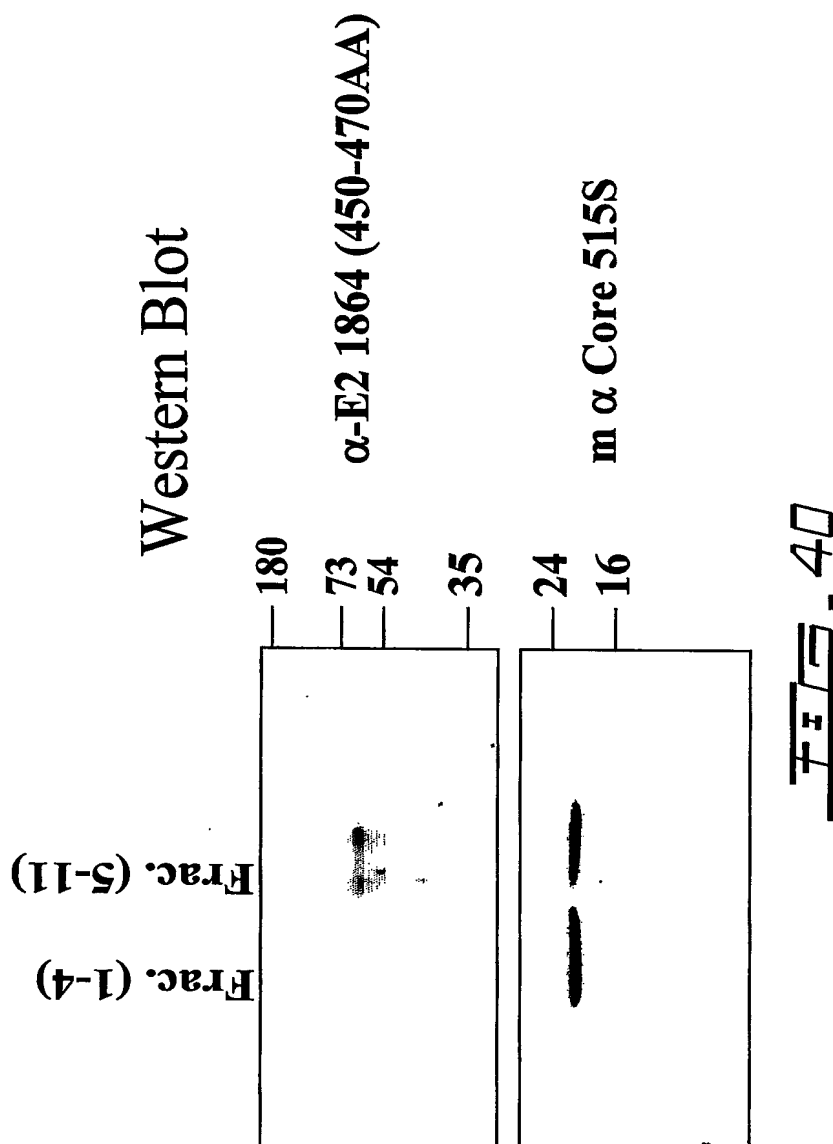
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Density Range (g/ml)	Source	Reference
1.15-1.20	HCV-LP in VSV vector	J.Virol (2002) 76, 12325.
1.14-1.18	HCV-LP in insect cells	J. Virol (1998) 72, 3827.
1.12-1.17	Plasma chimps	J. Gen.Virol (1994) 75, 1755
1.09-1.21	Plasma chimps	J.Med.Virol (1991), 34, 206.
1.13-1.17	Plasma chimps	J.Virol (1993) 67, 1953
1.063-1.21	Serum infected donors	J Med Virol (2002) 68, 335

1.11-1.215	HCV(+) PBMCs	-----
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FIG. 39

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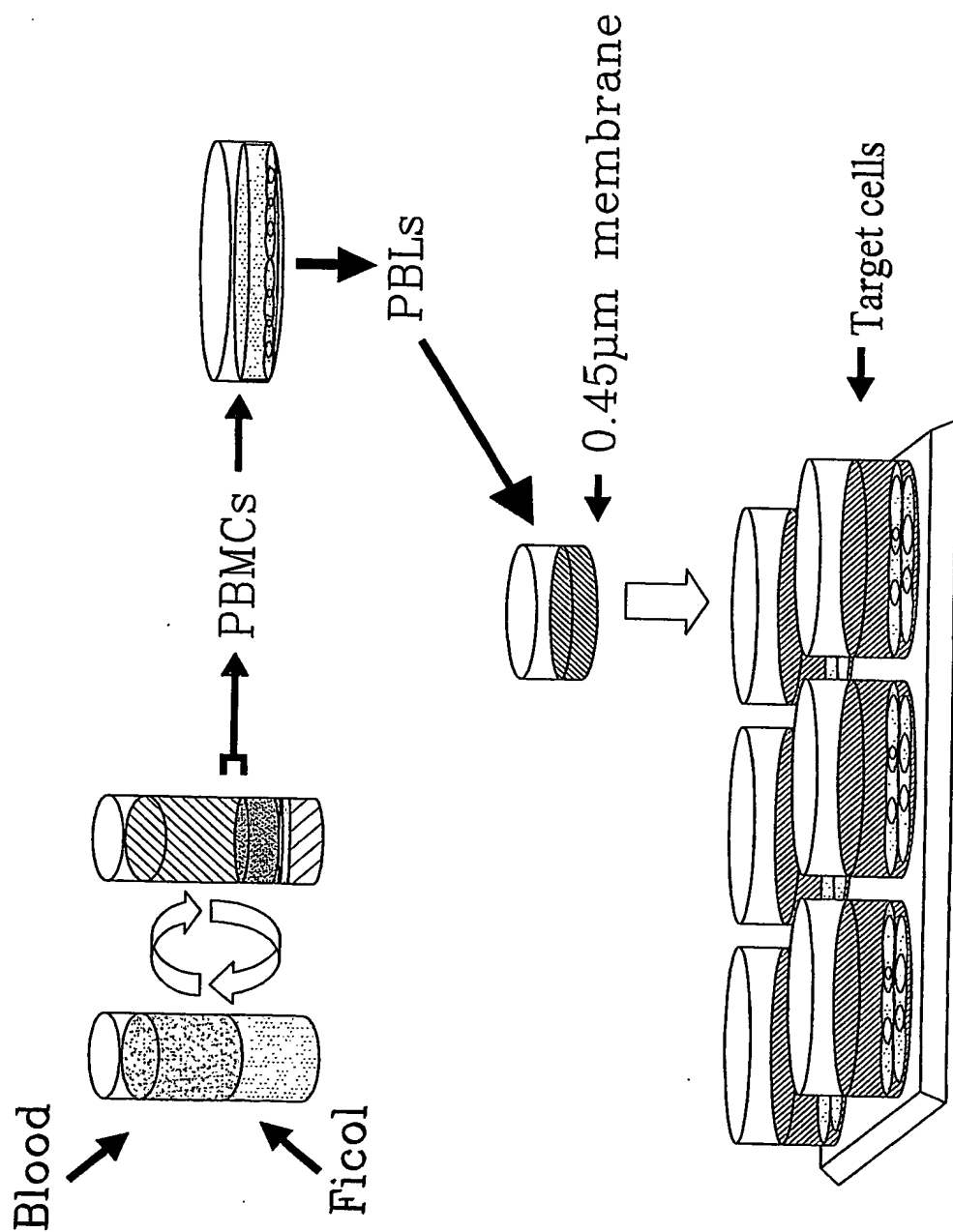
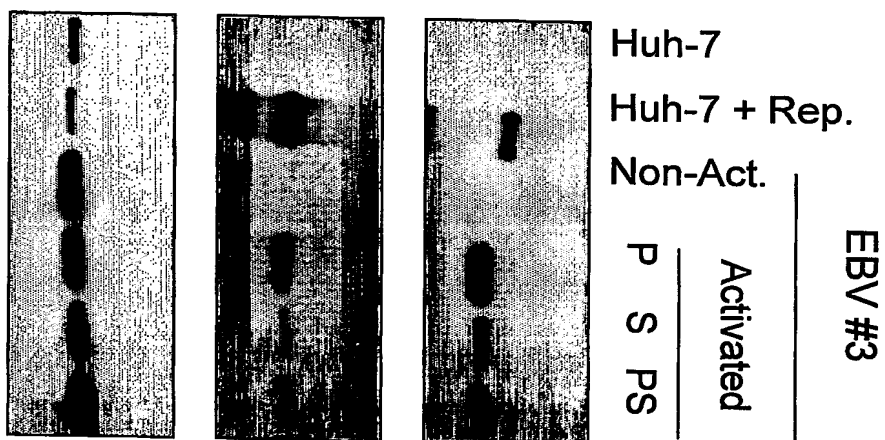
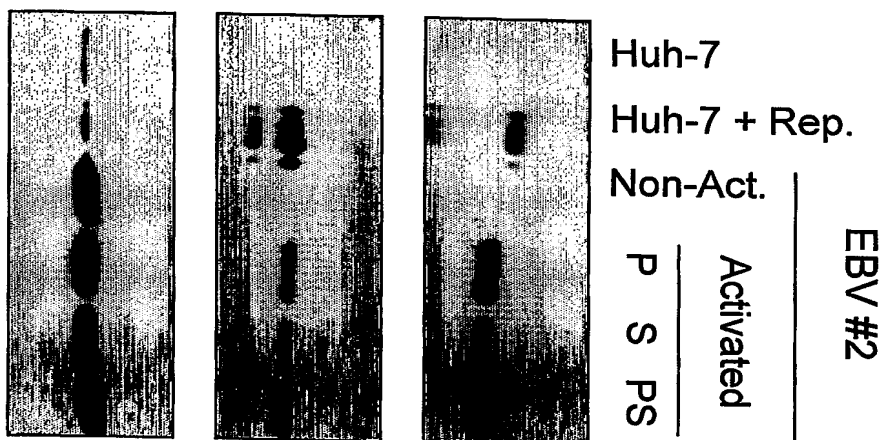
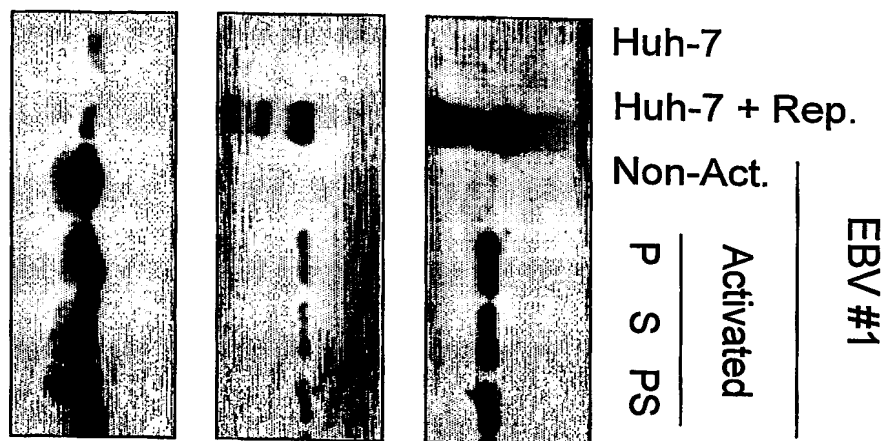


FIG. 41

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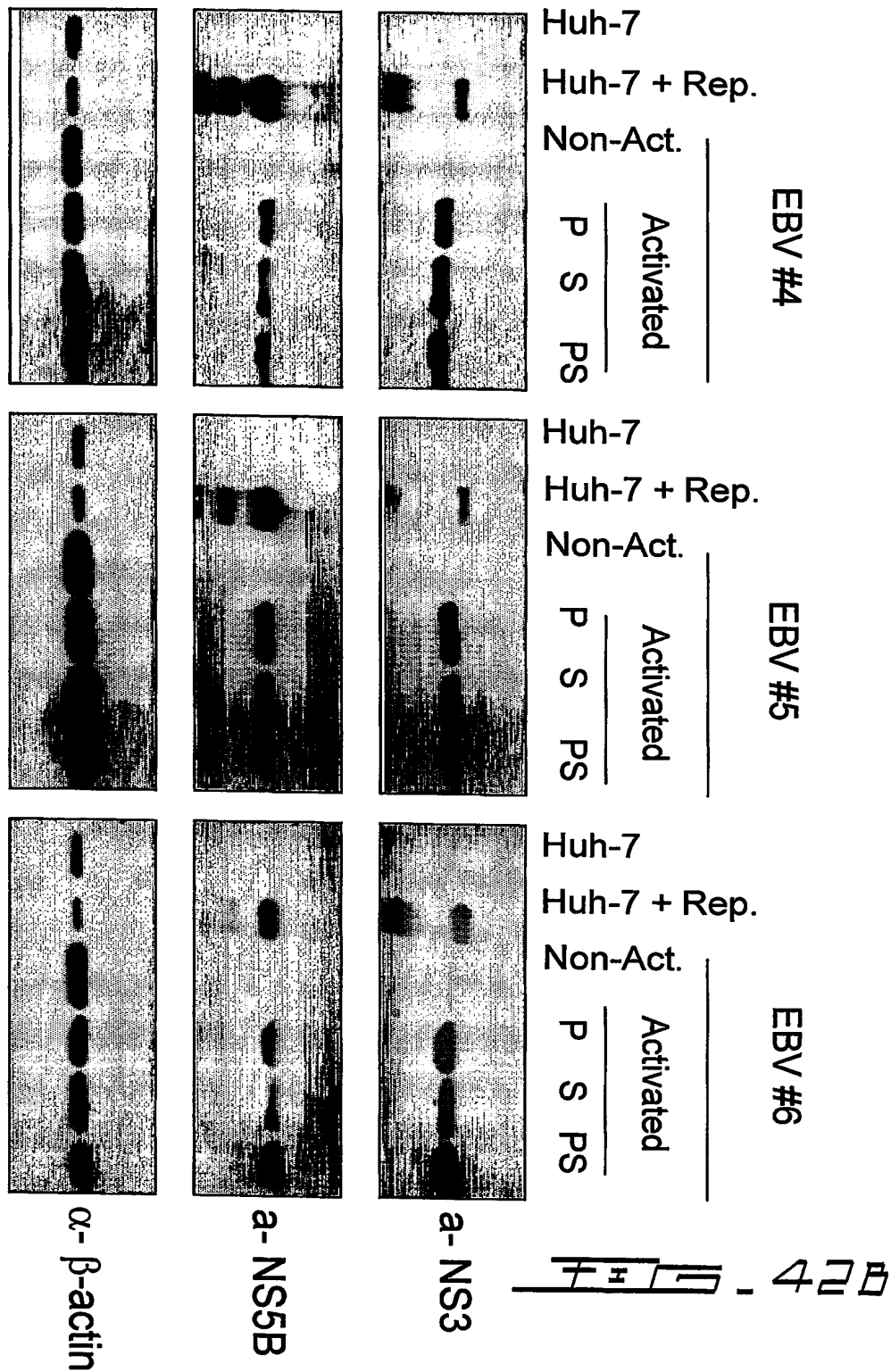
$\alpha$ - $\beta$ -actin

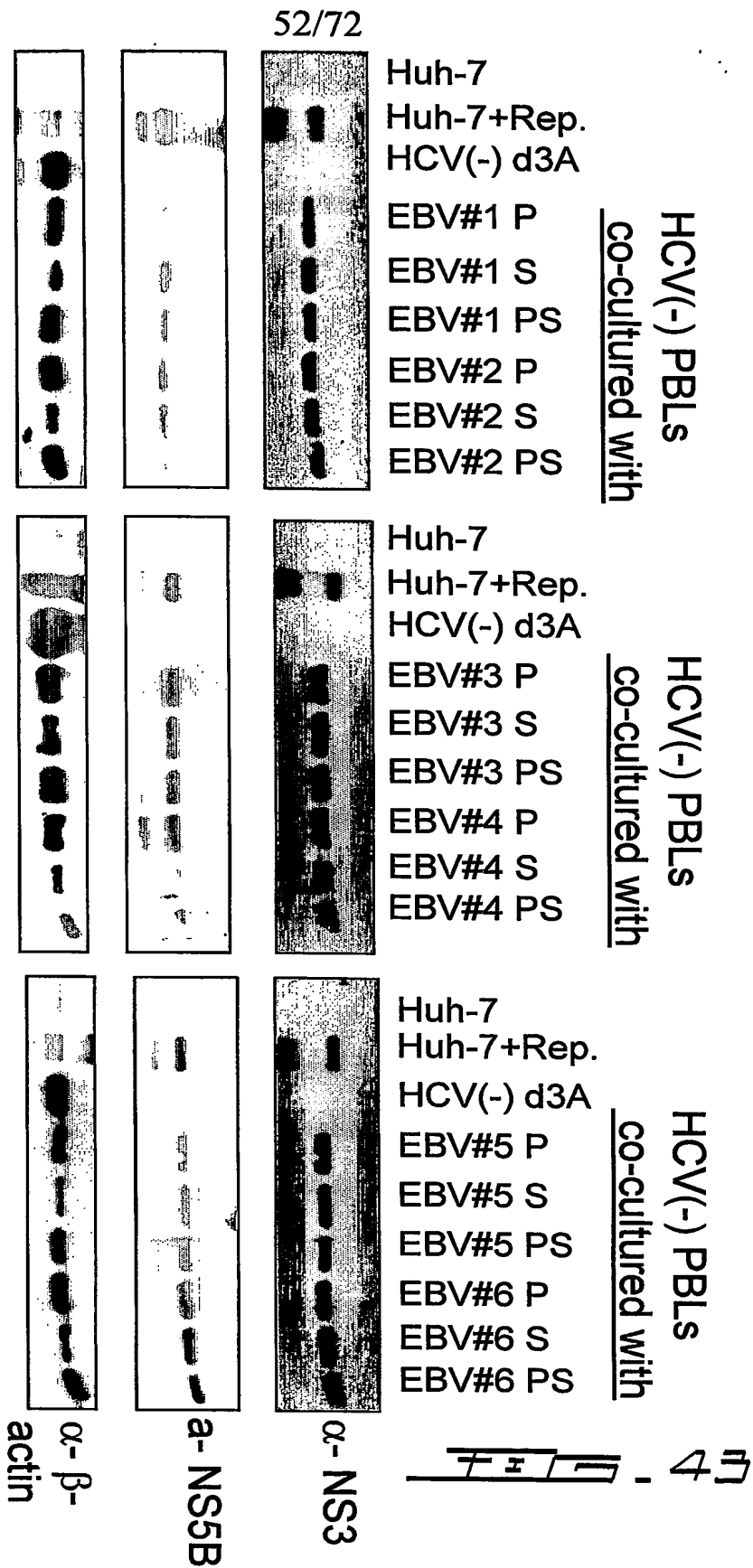
$\alpha$ -NS5B

$\alpha$ -NS3

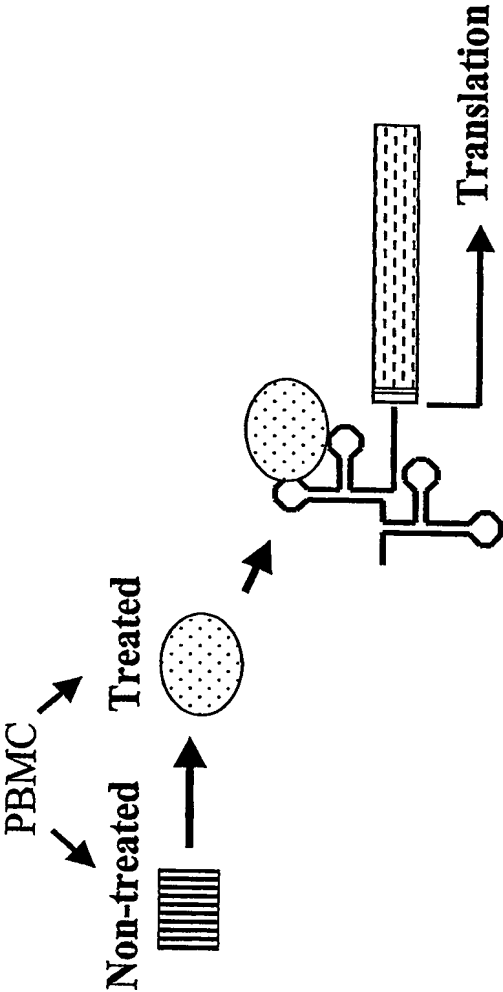
7.42A

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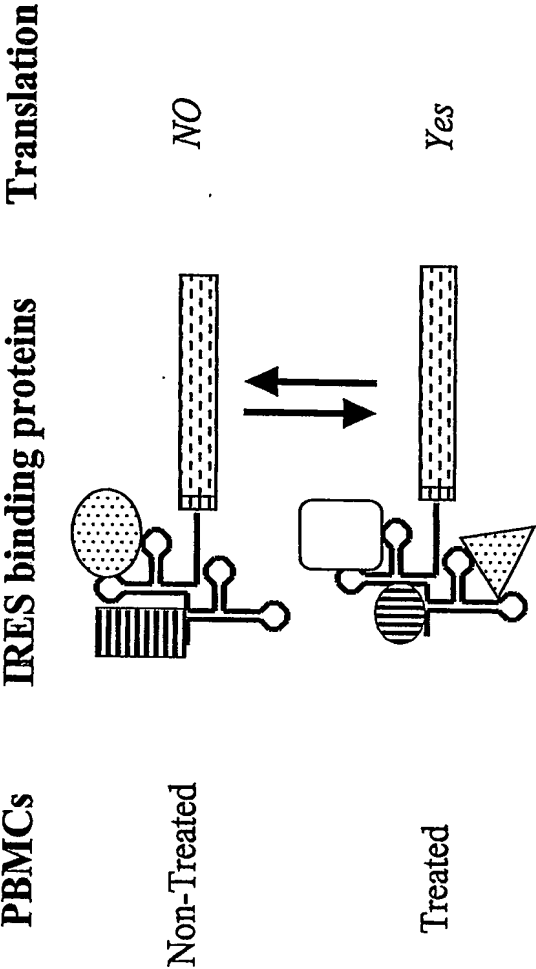




I- Translation Activator.



II- Translation inhibitor.



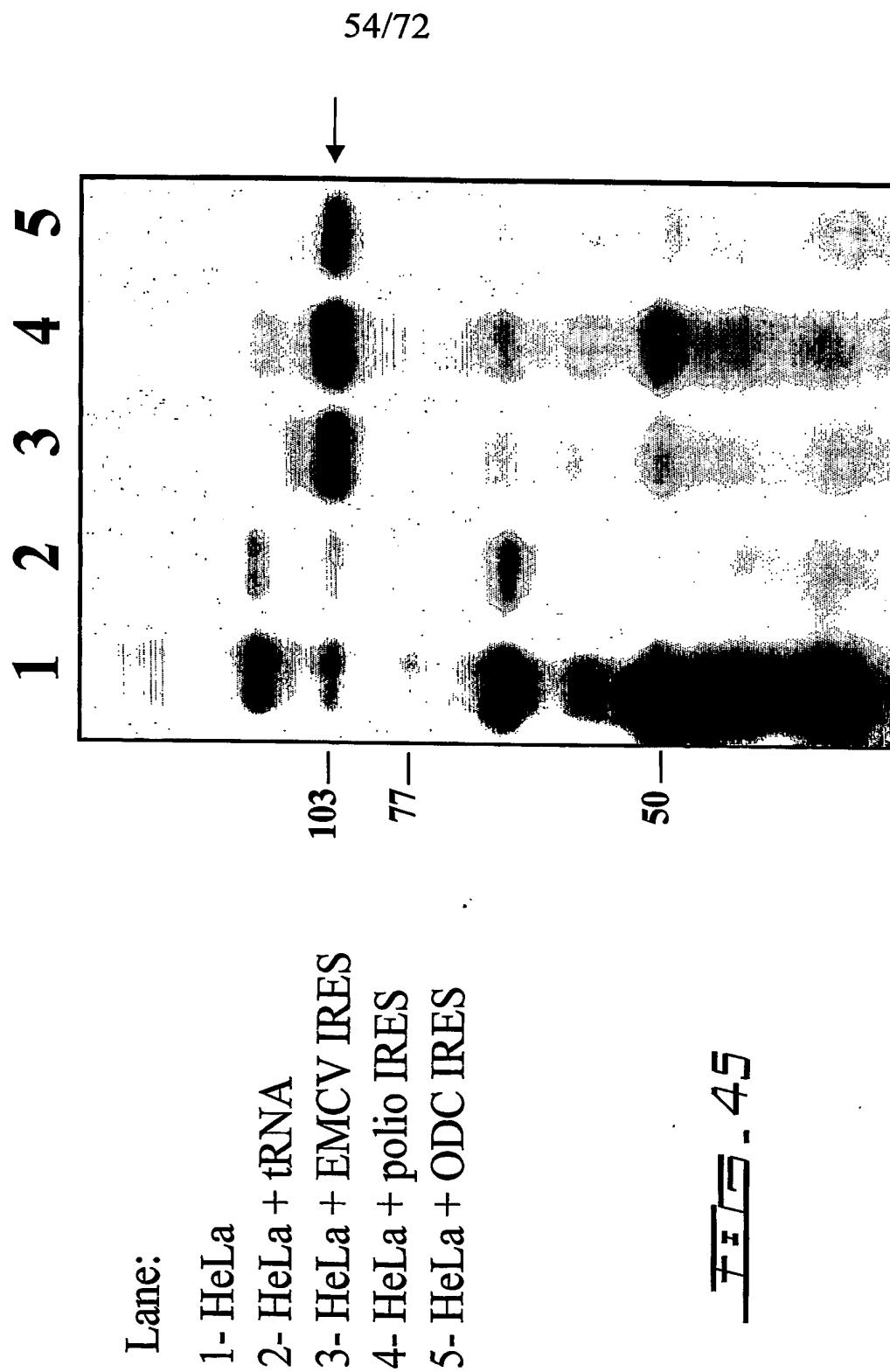
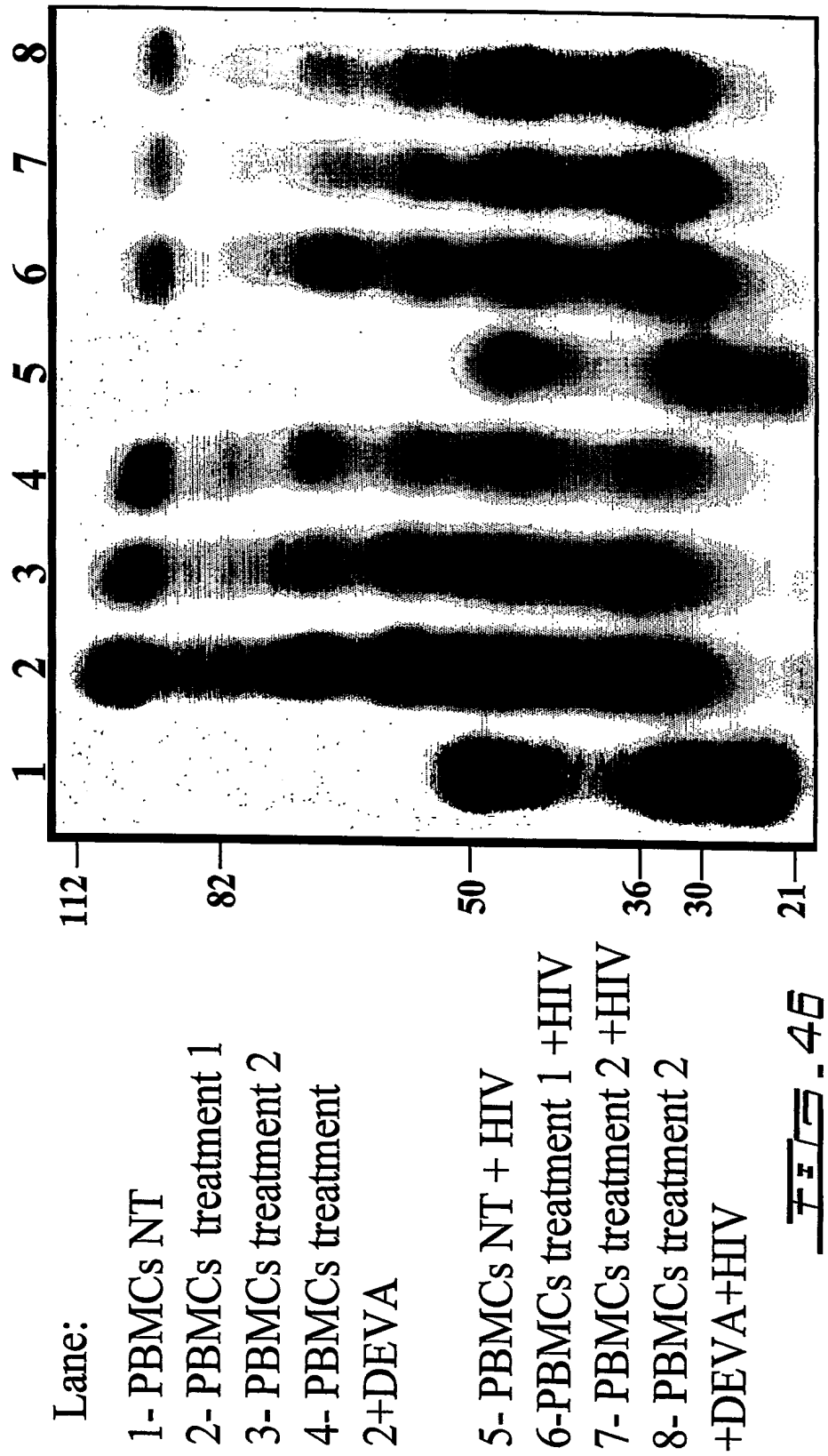
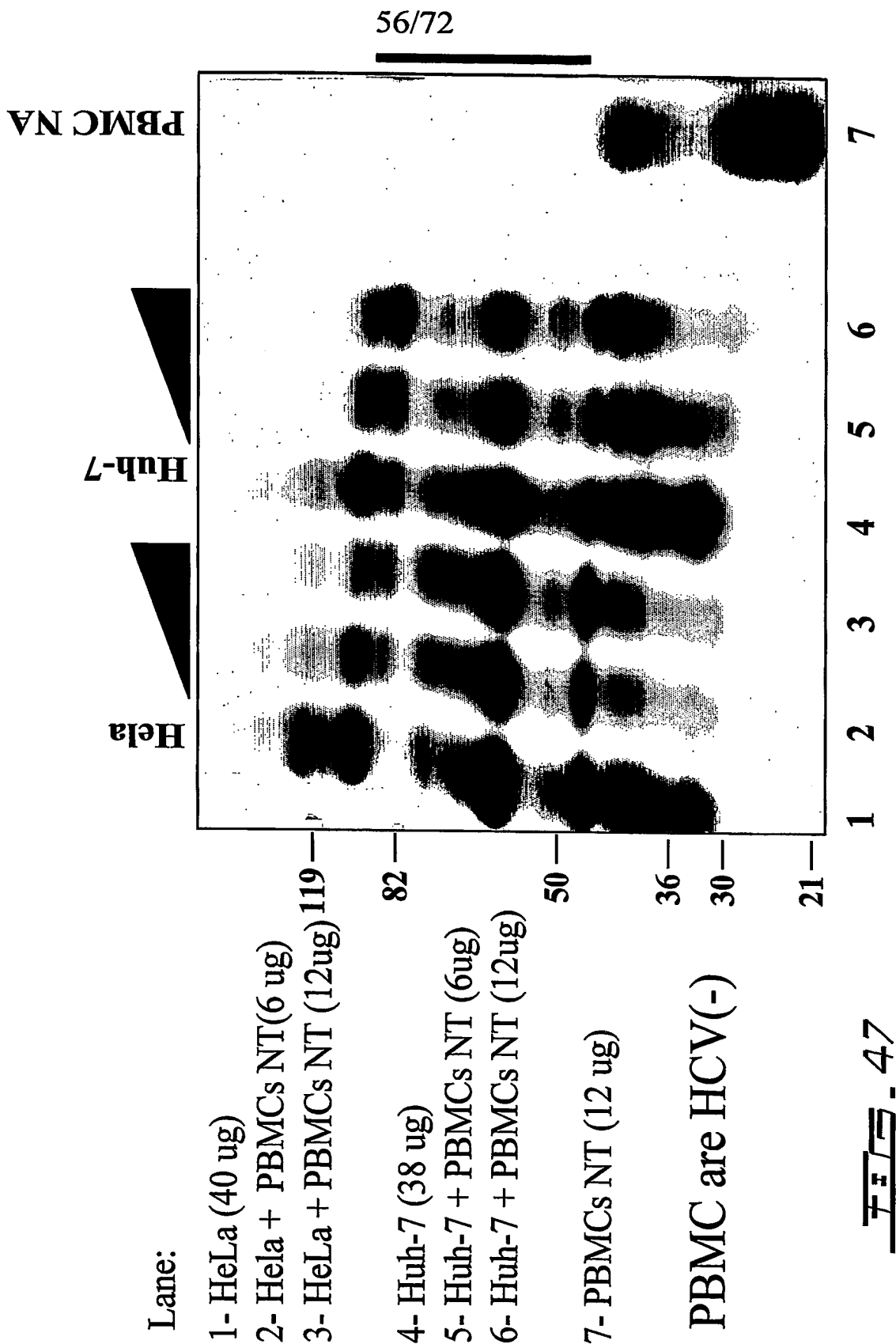


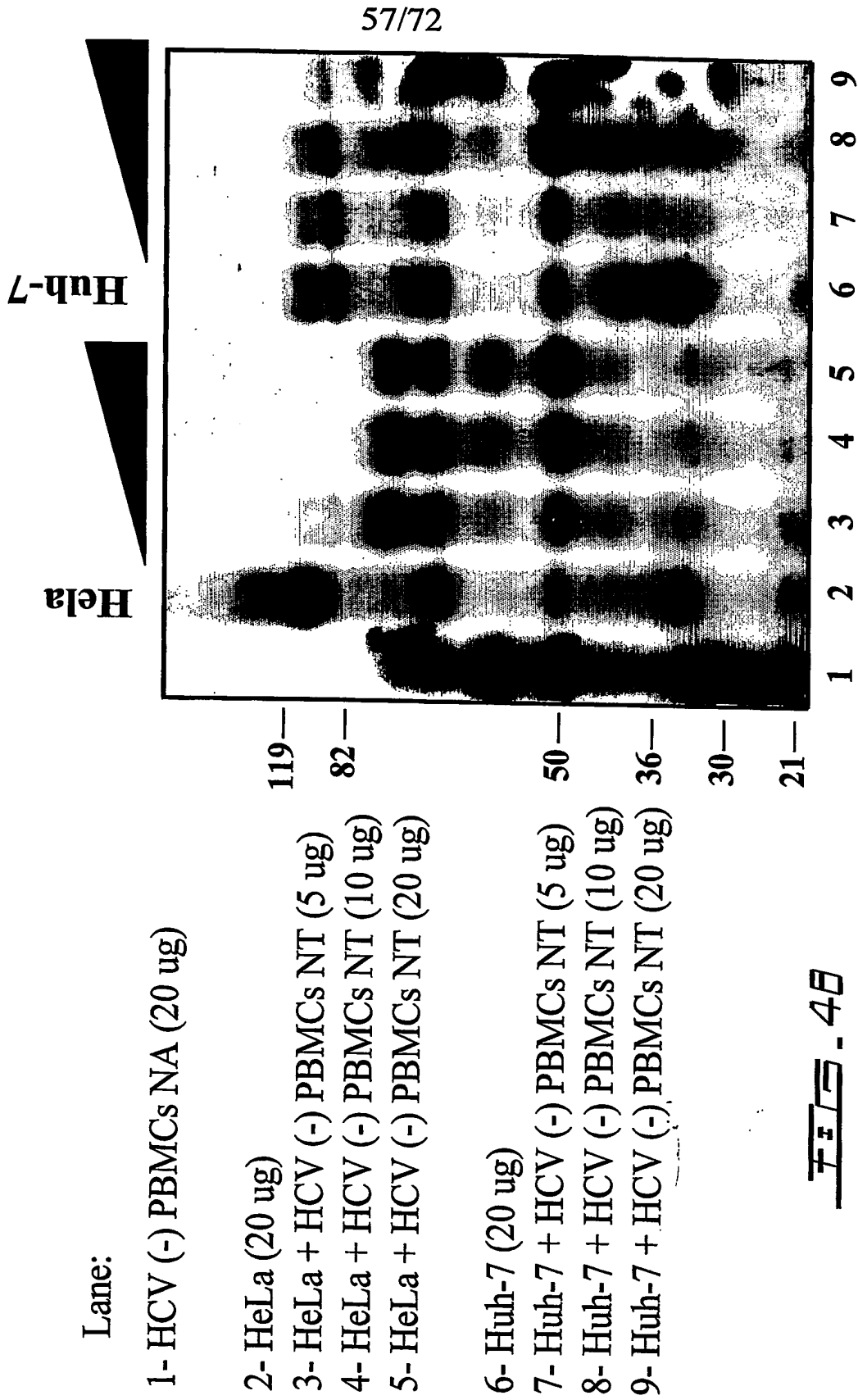
Fig. 45

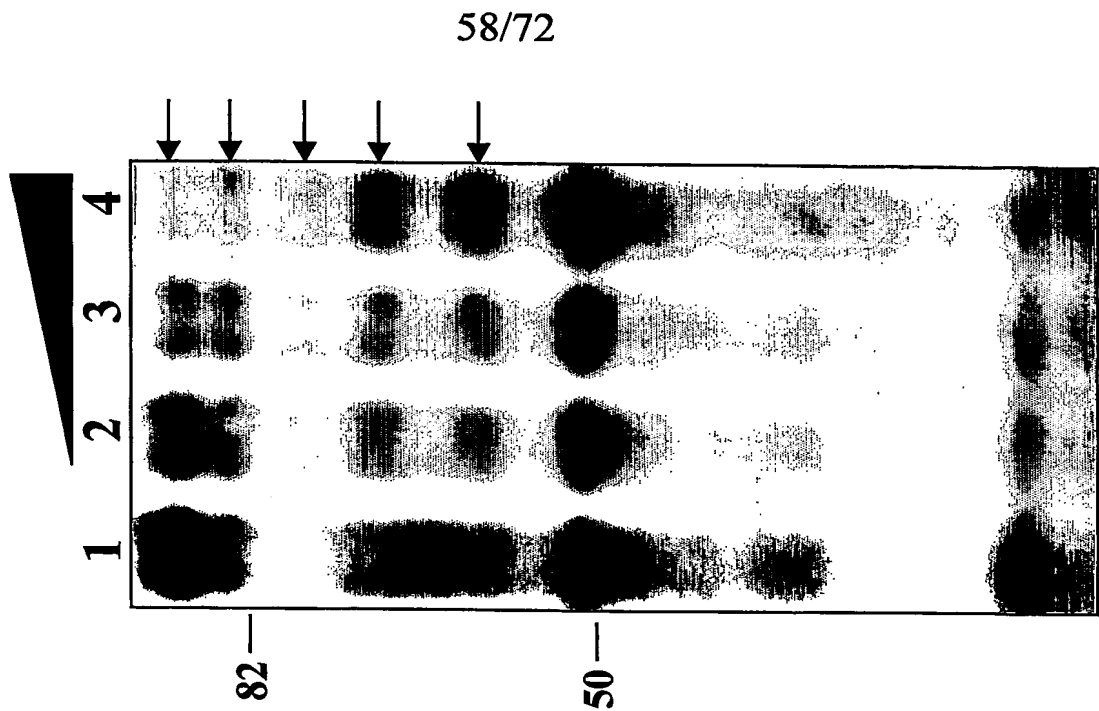
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Lane:

1- Huh-7 (20ug)

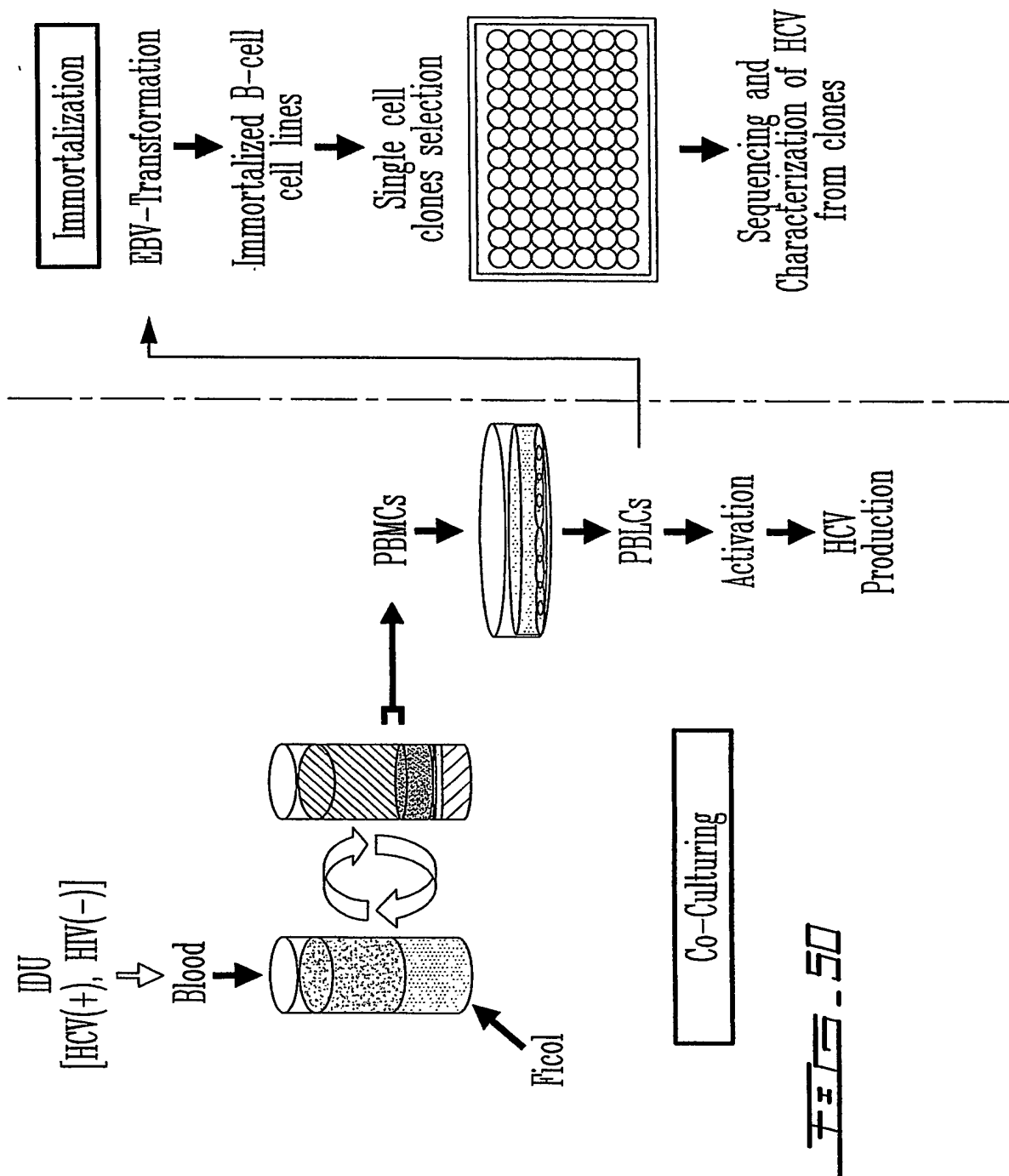
2- Huh-7 + HCV (-) PBMCs NT (5ug)

3- Huh-7 + HCV (-) PBMCs NT (10ug)

4- Huh-7 + HCV (-) PBMCs NT (20ug)

FIG. 49

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FIG. 50

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# HCV(+)- EBV-Transformed B-Cells.

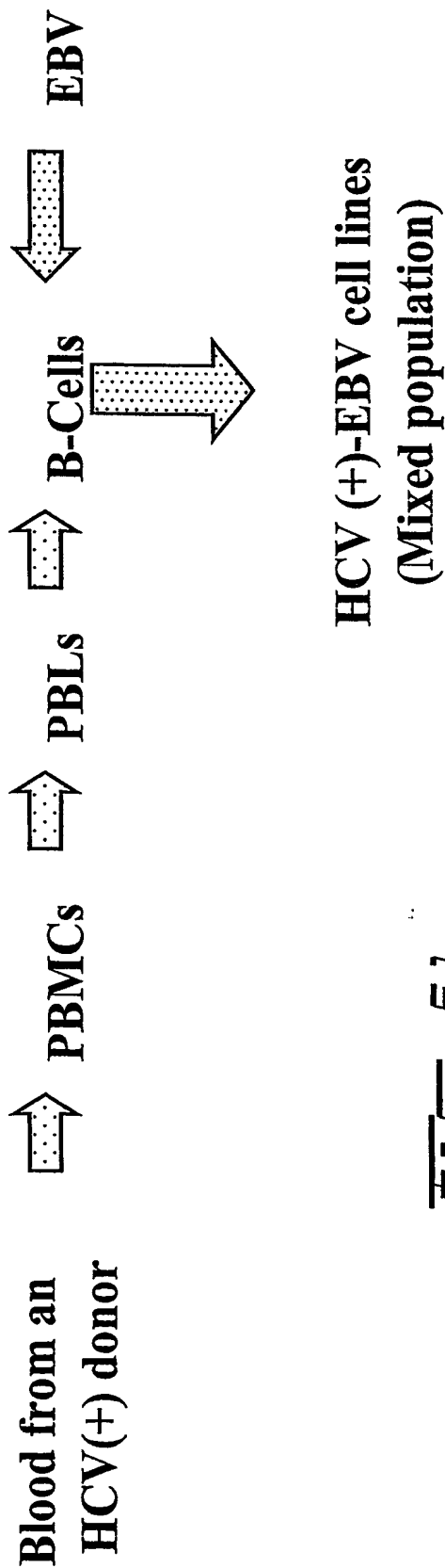


FIG. 51

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HCV RNA is detected in mixed population of EBV-transformed B-cells

### HCV (+) Strand RNA

	Non-Stimulated cells	Stimulated cells
Cell line	RNA Copies /10 <sup>6</sup> cells	RNA Copies /10 <sup>6</sup> cells
EBV-1	4.66x10 <sup>5</sup>	2.33x10 <sup>6</sup>
EBV-2	2.77x10 <sup>5</sup>	7.91x10 <sup>4</sup>
EBV-3	3.96x10 <sup>6</sup>	4.02x10 <sup>5</sup>
EBV-4	2.03x10 <sup>6</sup>	1.57x10 <sup>6</sup>
EBV-6	1.41x10 <sup>6</sup>	4.32x10 <sup>5</sup>
EBV-HCV (-)	0	0

### GAPDH mRNA

	Non-Stimulated cells	Stimulated cells
Cell line	RNA Copies /10 <sup>6</sup> cells	RNA Copies /10 <sup>6</sup> cells
EBV-1	2.23x10 <sup>8</sup>	2.19x10 <sup>8</sup>
EBV-2	8.73x10 <sup>8</sup>	2.25x10 <sup>8</sup>
EBV-3	1.83x10 <sup>9</sup>	1.77x10 <sup>9</sup>
EBV-4	5.48x10 <sup>8</sup>	3.79x10 <sup>8</sup>
EBV-6	1.26x10 <sup>9</sup>	9.42x10 <sup>8</sup>
EBV-HCV (-)	9.27x10 <sup>7</sup>	3.62x10 <sup>8</sup>

FIG - 52

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Control EBV-HCV (-); anti-Core

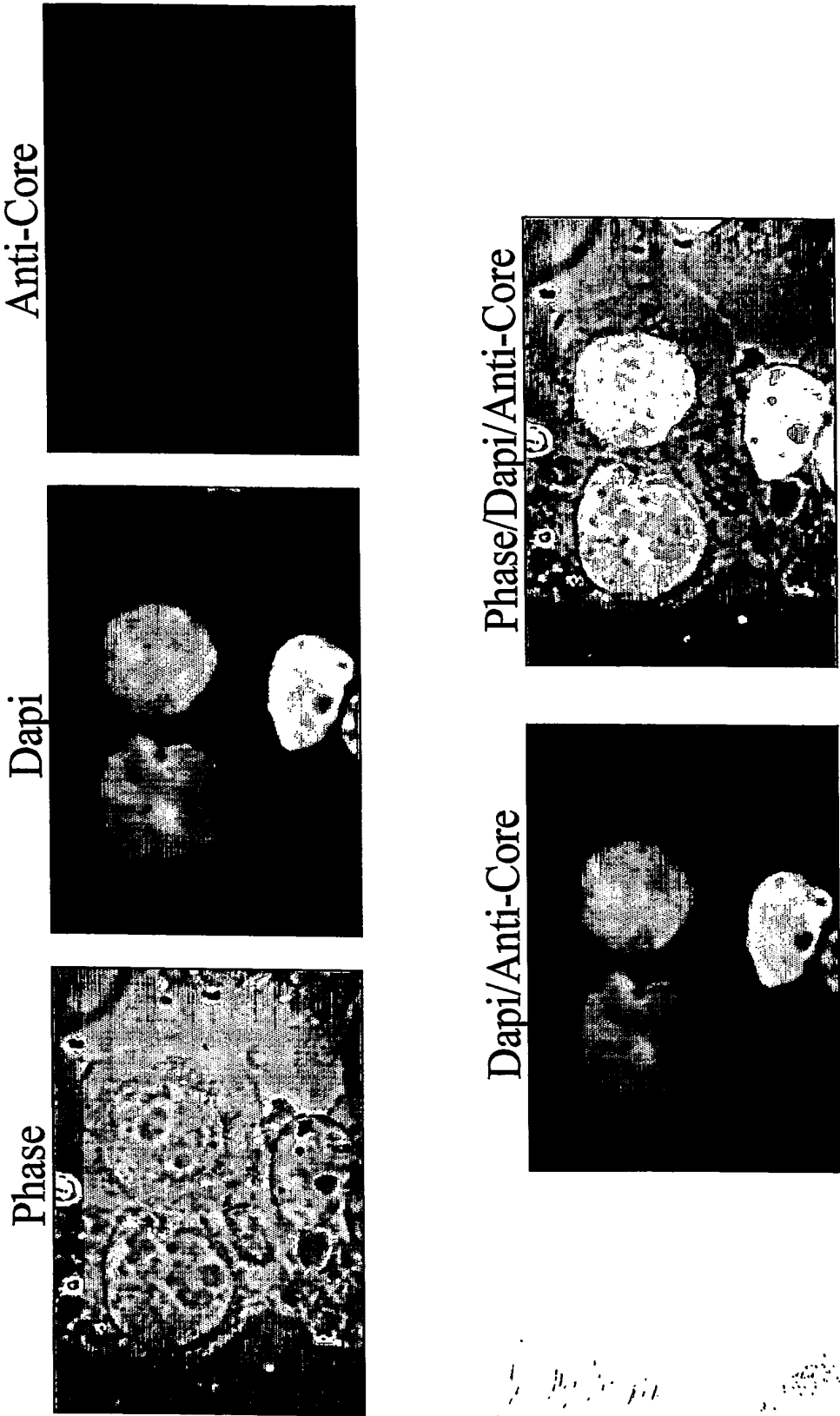


FIG. 53A

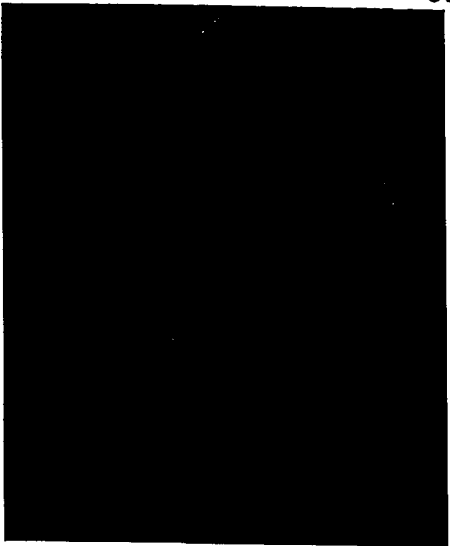
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Detection of Core in EBV-2

Dapi



Anti-Core



Dapi/Anti-Core



Phase/Dapi/Anti-Core



FIG. 53B

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# HCV(+)-EBV-Transformed B-Cells.

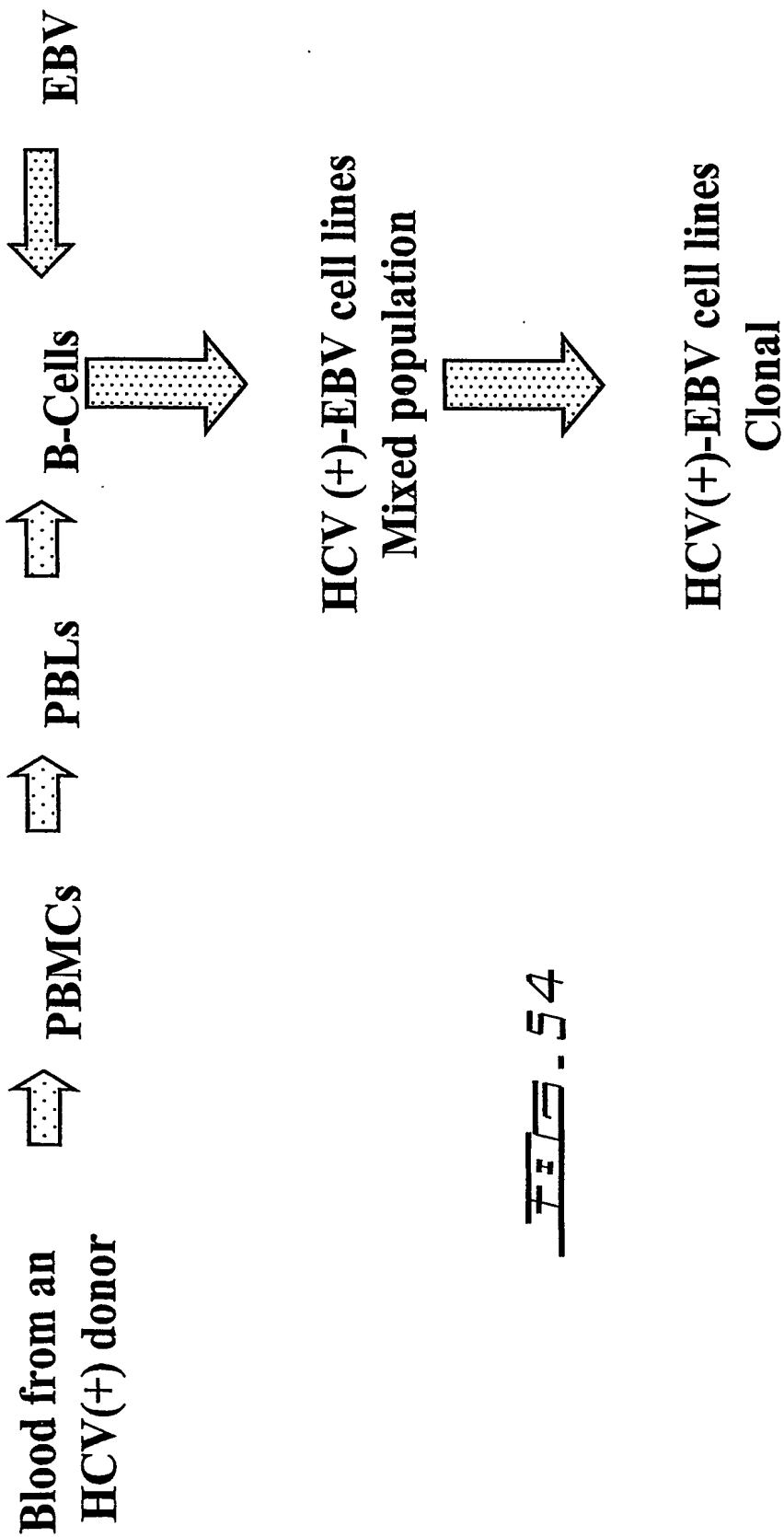
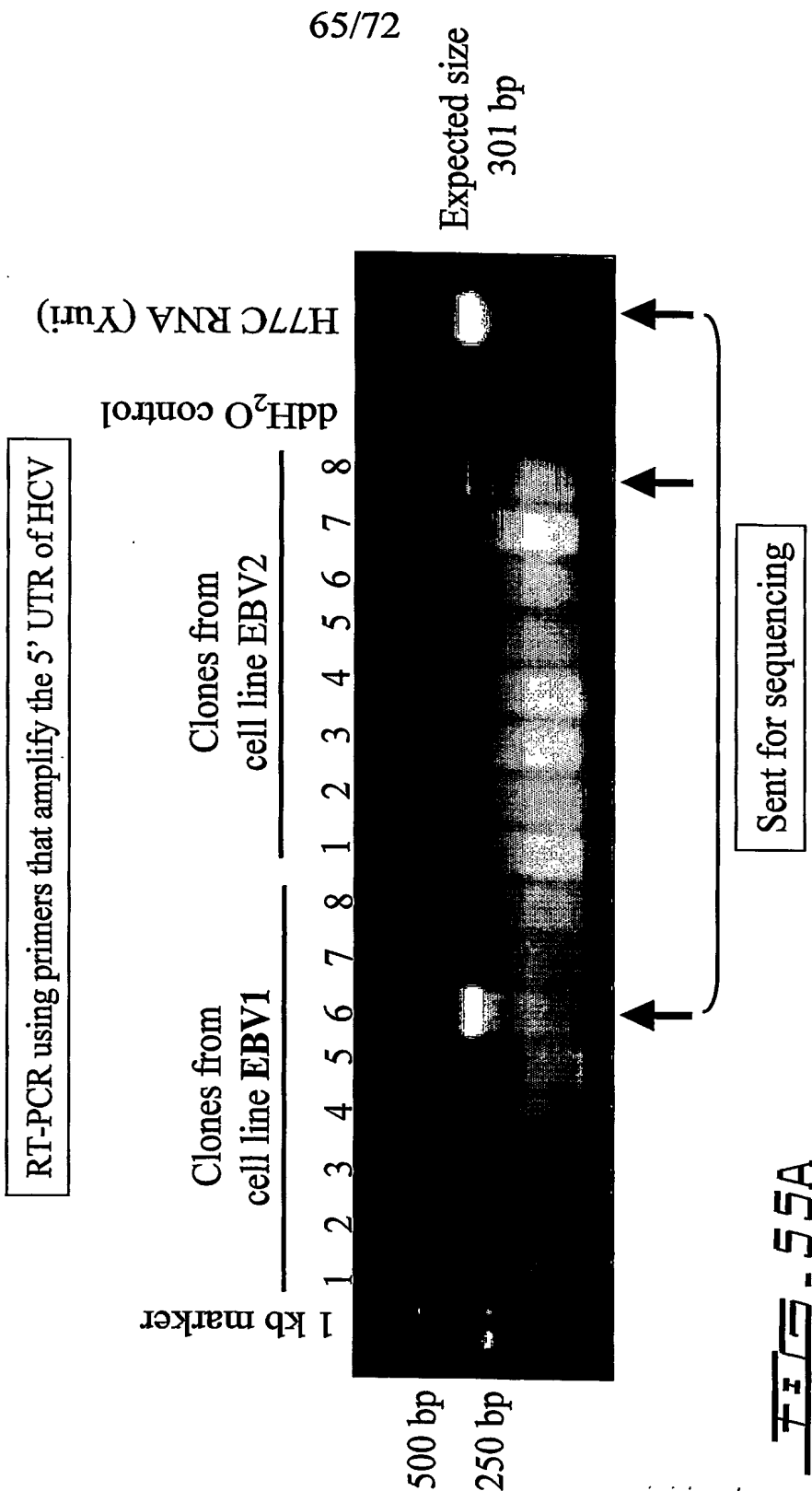


FIG. 54





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Alignment: H77C (RT-PCR positive control) sequence (top)/  
EBV1 clone 6 sequence (bottom)

```

CACTCCCCGTGAGGACTACTGTCTTCACGCAGAAAGCGTCTAGCCATGGCGT
CACTCCCCGTGAGGACTACTGTCTTCACGCAGAAAGCGTCTAGCCATGGCGT

TAGTATGAGTGTGTCAGCCTCCAGGACCCCCCTCCCGGAGAGCCATAGTGGTC
TAGTATGAGTGTGTCAGCCTCCAGGACCCCCCTCCCGGAGAGCCATAGTGGTC

      G

TGCGGAACCGGTGAGTACACCGGAATTGCCAGGACGACCGGGTCCTTCTTGGATAA
TGCGGAACCGGTGAGTACACCGGAATTGCCAGGACGACCGGGTCCTTCTTGGATAA

ACCCGCTCACATGCCTGGAGATTGGGCGTGCCCCCGCAAGACTGCTAGCCGAGTAG
ACCCGCTCA -ATGCCCTGGAGATTGGGCGTGCCCCCGCGGAGACTGCTAGCCGAGTAG

TGTTGGGTCGCGAAAGGCCCTTGTGGTACTGCCTGATAGGGT
TGTTGGGTCGCGAAAGGCCCTTGTGGTACTGCCTGATAGGGT

```

Fig. 55B

Blue: sequence from virus in the serum (MLL-005).

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Alignment: H77C (RT-PCR positive control) sequence (top)/  
EBV2 clone 8 sequence (bottom).

CCAGGACCCCTCCCGGAGAGCCATAGTGGTCTGCGGAACC  
CCAGGACCCCTCCCGGAGAGCCATAGTGGTCTGCGGAACC

GGTGATACACCGGAATTGCCAGGACCGGTCCTTTCTTGG  
GGTGATACACCGGAATTGCCAGGACCGGTCCTTTCTTGG

ATAAACCCGCTCAATGCCCTGGAGATTTGGGCGTGCCCCCGCAAG  
ATAAATCCGCTCAATGCCCTGGAGATTTGGGCGTGCCCCCGCAAG

ACTGCTAGCCGAGTAGTGTTGGTCCGAAAGGCCTTGTGGTAC  
ACTGCTAGCCGAGTAGTGTTGGTCCGAAAGGCCTTGTGGTAC

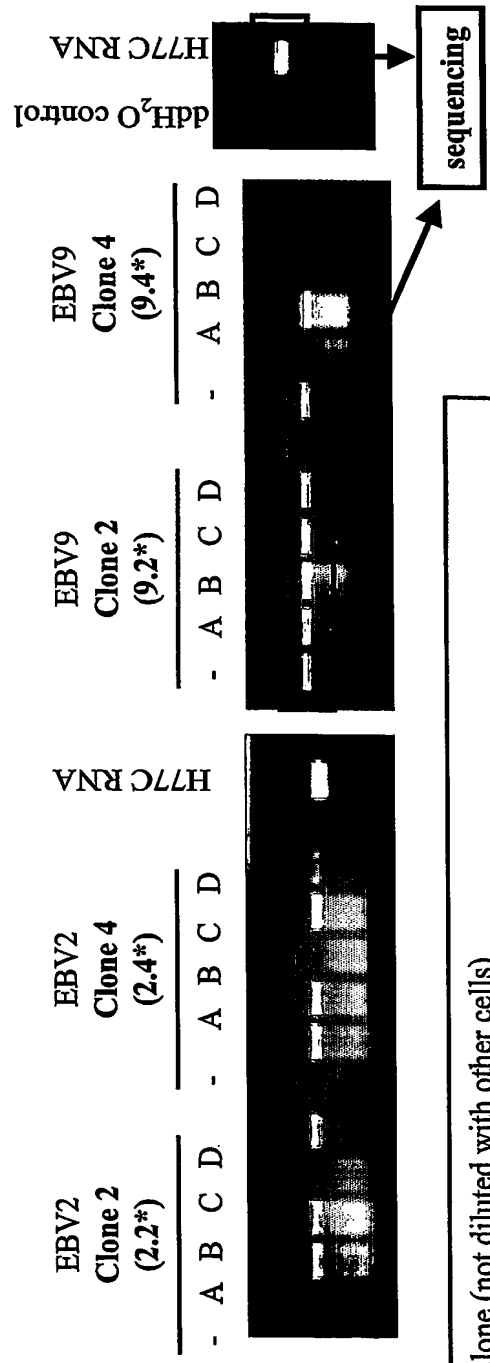
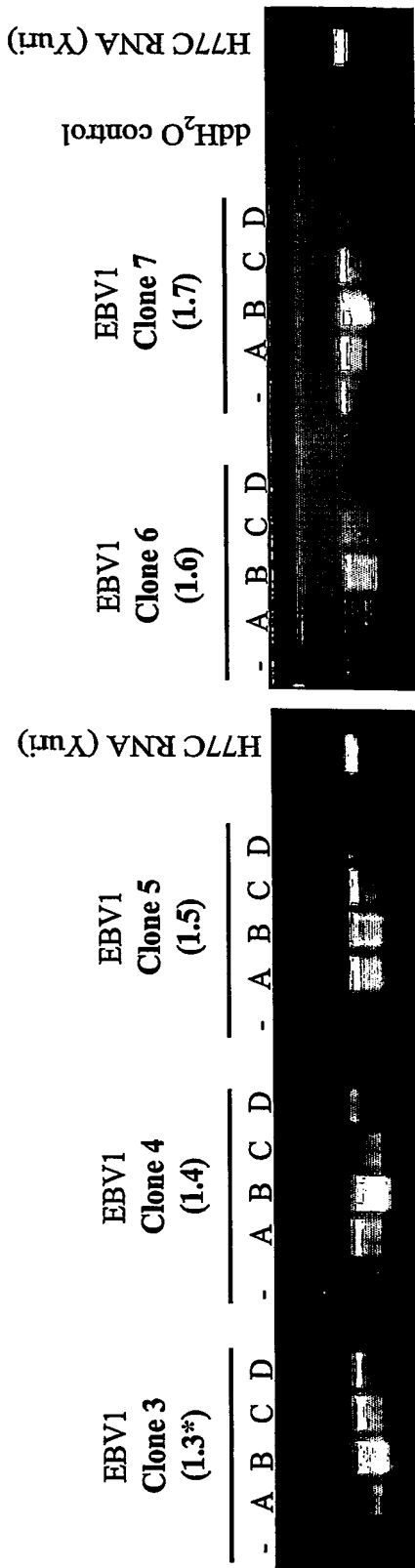
TGCCTGATAGGTGCTTGCGAGTGCCCCGGAGGTCTCGTAGAC  
TGCCTGATAGGTGCTTGCGAGTGCTCCGGGAGGTCTCGTAGAC

CGTGCA

CGTGCA

FIG - 55C

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- = clone alone (not diluted with other cells)  
A = diluted 1:10 with MT4 cell line (HTLV1 transformed T cells)  
B = diluted 1:10 with BJAB cell line (ATCC non-EBV transformed B cells)  
C = diluted 1:10 with HLA 006 cell line (EBV transformed HCV- PBLs)  
D = diluted 1:10 with JAM cell line (EBV transformed HCV- PBLs)

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Alignment of all 9.2 sequences

H77C CACTCCCCTGTGAGGAACTACTGTCTTACGCAGAAAGCGTCT  
9.2 final seq CACTCCCCTGTGAGGAACTACTGTCTTACGCAGAAAGCGTCT  
9.2a final seq CACTCCCCTGTGAGGAACTACTGTCTTACGCAGAAAGCGTCT  
9.2b final seq CACTCCCCTGTGAGGAACTACTGTCTTACGCAGAAAGCGTCT  
9.2c final seq CACTCCCCTGTGAGGAACTACTGTCTTACGCAGAAAGCGTCT  
9.2d final seq CACTCCCCTGTGAGGAACTACTGTCTTACGCAGAAAGCGTCT

H77C AGCCATGGCGTTAGTATGAGTGTCTGCAGCCTCCAGGACCCCC  
9.2 final seq AGCCATGGCGTTAGTATGAGTGTCTGCAGCCTCCAGGACCCCC  
9.2a final seq AGCCATGGCGTTAGTATGAGTGTCTGCAGCCTCCAGGACCCCC  
9.2b final seq AGCCATGGCGTTAGTATGAGTGTCTGCAGCCTCCAGGACCCCC  
9.2c final seq AGCCATGGCGTTAGTATGAGTGTCTGCAGCCTCCAGGACCCCC  
9.2d final seq AGCCATGGCGTTAGTATGAGTGTCTGCAGCCTCCAGGACCCCC

H77C CCTCCCGGGAGAGCCATAGTGGTCTCGGAAACCGGTAGTACAC  
9.2 final seq CCTCCCGGGAGAGCCATAGTGGTCTCGGAAACCGGTAGTACAC  
9.2a final seq CCTCCCGGGAGAGCCATAGTGGTCTCGGAAACCGGTAGTACAC  
9.2b final seq CCTCCCGGGAGAGCCATAGTGGTCTCGGAAACCGGTAGTACAC  
9.2c final seq CCTCCCGGGAGAGCCATAGTGGTCTCGGAAACCGGTAGTACAC  
9.2d final seq CCTCCCGGGAGAGCCATAGTGGTCTCGGAAACCGGTAGTACAC

- = clone alone (not diluted with other cells)  
a= diluted 1:10 with MT4 cell line (HTLV1 transformed T cells)  
b= diluted 1:10 with BJAB cell line (ATCC non-EBV transformed B cells)  
c= diluted 1:10 with HLA 006 cell line (EBV transformed HCV- PBLs)  
d= diluted 1:10 with JAM cell line (EBV transformed HCV- PBLs)

Red= Variation with respect to clone 9.2

~~7375~~ - 57A

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# Alignment of all 9.2 sequences

H77C	CGGAATTGCCAGGACGACCGGGTCCTTCTTTGGATAAACCCGCT
9.2 final seq	CGGAATTGCCAGGACGACCGGGTCCTTCTTTGGATAAACCCGCT
9.2a final seq	CGGAATTGCCAGGACGACCGGGTCCTTCTTTGGAT <u>T</u> AACCCGCT
<b>9.2b final seq</b>	CGGAATTGCC <u>G</u> GA <u>A</u> GAC <u>T</u> GGGTCCTTCTTTGGATAAACCC <u>A</u> CT
9.2c final seq	CGGAATTGCCAGGACGACCGGGTCCTTCTTTGGATAAACCCGCT
9.2d final seq	CGGAATTGCCAGGACGACCGGGTCCTTCTTTGGAT <u>T</u> AATCCGCT
H77C	CAATGCCTGGAGATTTGGCGGTGCCCCCGCAAGACTGCTAGCCG
9.2 final seq	CAATGCCTGGAGATTTGGCGGTGCCCCCGCAAGACTGCTAGCCG
9.2a final seq	CAATGCCTGGAGATTTGGCGGTGCCCCCGC <u>G</u> AGACTGCTAGCCG
<b>9.2b final seq</b>	<u>C</u> <u>T</u> A <u>T</u> GCCCGG <u>C</u> C <u>A</u> TTTGGGCGTGCCCCCGCAAGACTGCTAGCCG
9.2c final seq	CAATGCCTGGAGATTTGGGCGTGCCCCCGCAAGACTGCTAGCCG
9.2d final seq	CAATGCCTGGAGATTTGGGCGTGCCCCCGC <u>G</u> AGACTGCTAGCCG

FIG. 57B

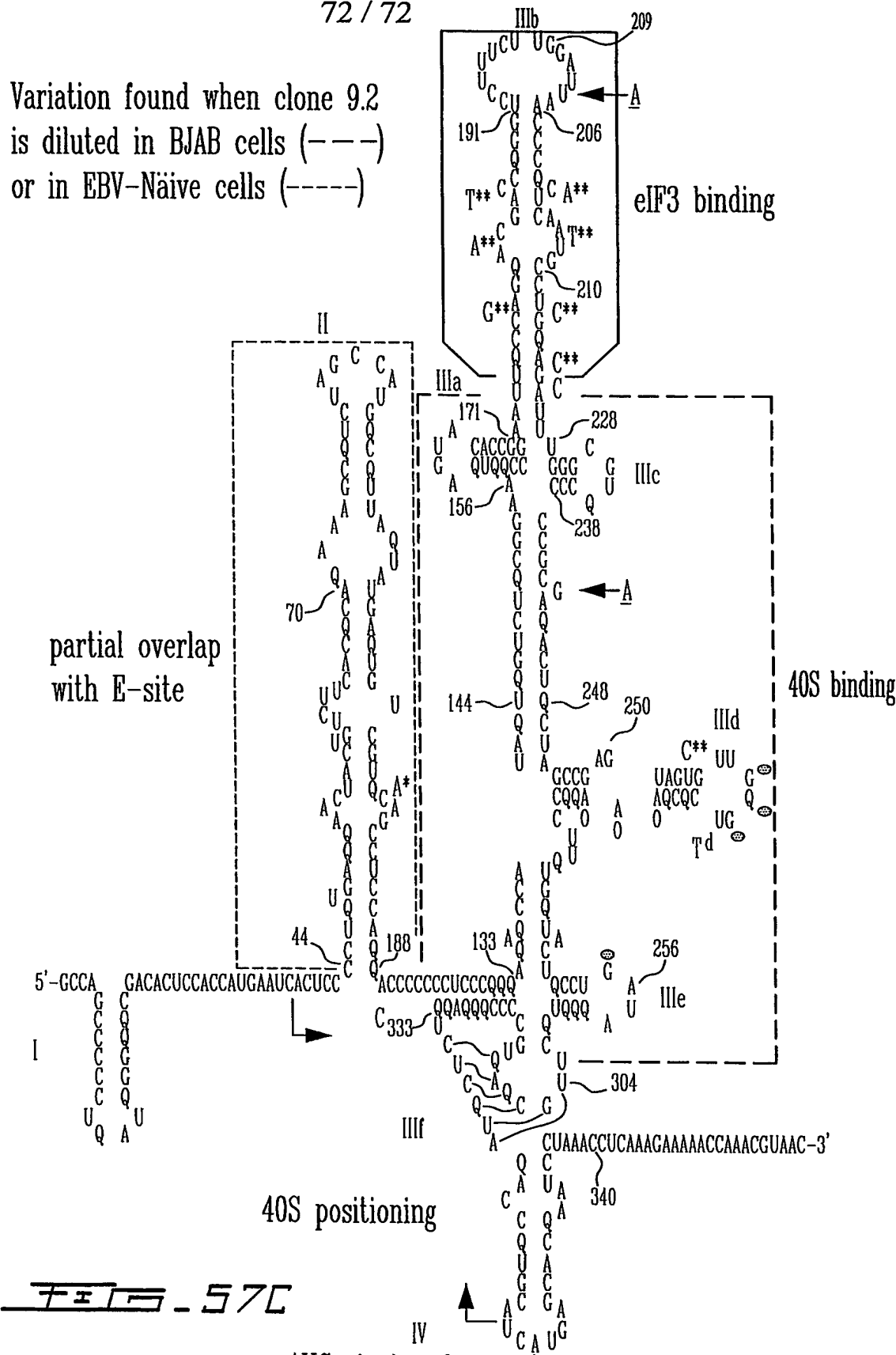
Alignment of all 9.2 sequences

H77C	AGTAGTGTGGTTCGCCGAAAGGCCCTTGTGGTACTGCCCTGATAGG
9.2 final seq	AGTAGTGTGGTTCGCCGAAAGGCCCTTGTGGTACTGCCCTGATAGG
9.2a final seq	AGTAGTGTGGTTCGCCGAAAGGCCCTTGTGGTACTGCCCTGATAGG
9.2b final seq	AGTAGCCTGGTTCGCCGAAAGGCCCTTGTGGTACTGCCCTGATAGG
9.2c final seq	AGTAGTGTGGTTCGCCGAAAGGCCCTTGTGGTACTGCCCTGATAGG
9.2d final seq	AGTAGTGTGGTTCGCCGAAAGGCCCTTGTGGTACTGCCCTGATAGG
H77C	GTGCTTGCGAGTGCCCCCGGAGGTCTCGTAGACCGTGCA
9.2 final seq	GTGCTTGCGAGTGCCCCCGGAGGTCTCGTAGACCGTGCA
9.2a final seq	GTGCTTGCGAGTGCCCCCGGAGGTCTCGTAGACCGTGCA
9.2b final seq	GTGCTTGCGAGTGCCCCCGGAGGTCTCGTAGACCGTGCA
9.2c final seq	GTGCTTGCGAGTGCCCCCGGAGGTCTCGTAGACCGTGCA
9.2d final seq	GTGCTTGCGAGTGCCCCCGGAGGTCTCGTAGACCGTGCA

FIG. 57B (Cont.)

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Variation found when clone 9.2  
is diluted in BJAB cells (---)  
or in EBV-Näive cells (-----)



➔ = Primers

AUG start codon  
IRES structure from Sarnow P (2003), J Virol. 77, 2801-6